EVALUATION OF MICROBIAL PROFILE IN DENTAL UNIT WATERLINES AND ASSESSMENT OF ANTIMICROBIAL EFFICACY OF TWO TREATING AGENTS

Dissertation submitted to

THE TAMILNADU Dr.M.G.R.MEDICAL UNIVERSITY

In partial fulfillment for the Degree of

MASTER OF DENTAL SURGERY

BRANCH VIII

PEDODONTICS & PREVENTIVE DENTISTRY

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CERTIFICATE

This is to certify that this dissertation titled " **Evaluation Of Microbial Profile In Dental Unit Waterlines And Assessment Of Antimicrobial Efficacy of Two Treating Agents"** is a bonafide record of work done by Dilna.N.C. under our guidance during the study period between 2006-2009.

This dissertation is submitted to THE TAMILNADU Dr.M.G.R. MEDICAL UNIVERSITY, in partial fulfillment for the degree of **MASTER OF DENTAL SURGERY –PEDODONTICS AND PREVENTIVE DENTISTRY, BRANCH VIII**. It has not been submitted (partial or full) for the award of any other degree or diploma.

Dr.M.Jayanthi,M.D.S
Professor &H.O.D
Department of Pedodontics & preventive dentistry
Ragas dental college & Hospital
Chennai

Dr.S.Ramachandran,M.D.S
Professor&Principal
Ragas dental college & Hospital
Chennai

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CONTENTS

INTRODUCTION

A successful treatment requires sterile environment which in turn renders infection control a major importance in routine daily procedures in the dental office. The goal of infection control is to prevent the spread of infection from one patient to another and to the treating health care worker. This can be achieved by a series of actions such as hand washing and gloving, protection against aerosol and splatter with the use of facemasks, eye wear, protective clothing and Instrument processing. It also includes surface asepsis, management of sharps and other waste products as well as Aseptic techniques. Many of the infection control measures called 'Universal precautions' [13] are recommended by national dental associations and for effective infection control, every possible source of contamination should be submitted to these actions before, during and after dental intervention.

The quality of water in a dental unit used for cooling and flushing the high and low speed handpiece, air/water syringes and the scalers is of considerable importance because patients and dental staff are regularly exposed to water and aerosol generated from dental unit . Source of water supply to these dental units, in the case of an open system is municipal water, while in the case of a closed system –water is poured into a reservoir belonging to a dental unit called as the independent water system , is an initial part of dental unit waterlines.

Transmission of microbial pathogens from biofilm within dental unit waterlines to the patients is a concern because it is difficult to maintain the sterilization

in those areas. Bacteria in natural aquatic environments have a marked tendency to interact with surfaces. The formation of surface biofilms can be regarded as a universal bacterial strategy for survival and for optimum positioning with regard to available nutrients.

The term 'biofilm' refers to the development of microbial communities on submerged surfaces in aqueous environment. The formation and growth of biofilm is considered to be as a result of complex processes involving transport of organic and inorganic molecules and microbial cells to the surface, adsorption of molecules to the surface and initial attachment of microbial cells followed by their irreversible adhesion, facilitated by production of extracellular polymeric substances, often referred as glycocalyx or slime.

Biofilm-associated organisms also differ from their planktonic (freely suspended) counterparts with respect to genes that are transcribed. Biofilms may form on a wide variety of surfaces, including living tissues, indwelling medical devices, industrial or portable water system piping, or a natural aquatic system. Donlan and Costerton[48] propose a new definition of biofilm as a " microbially-derived sessile community characterized by cells that are irreversibly attached to a substrate or to each other, are embedded in a matrix of extracellular polymeric substances that they have produced and exhibit an altered phenotype with respect to growth rate and gene transcription ."

Two problems can arise from the presence of biofilms in a distributing aqueous system. First, the biofilm can clog pipes and tubings or interfere with the proper function of mechanical devices. Second, bacterial populations living in this protected mode of growth

produce planktonic cells that contaminate fluids and alter their properties or, in the case of pathogens, can result in food poisoning or infections. In addition, biofilm bacteria are substantially resistant to surfactants, biocides, and antibiotics. As a result, microbial biofilms constitute major industrial and medical concerns. These concerns are now being realized in the dental profession.

The presence of microbial contamination of the water coming from dental units was first reported by Blake[5] in 1963. Since then the research has been ongoing to identify potential bacterial human pathogens from dental unit waterlines (DUWL) and also to assess the efficacy of different products and techniques to reduce, if not eliminate, microbial levels in dental unit water. A recommendation has been issued by the American Dental Association[1] that by the year 2000, water for non surgical procedures should contain no more than 200cfu/ml of aerobic, mesophilic, heterotrophic bacteria in the unfiltered output of dental unit waterline. The safety of dental treatment requires a good quality of the water used. The knowledge of formation and the ways to eliminate the biofilm is the first step towards reducing health risk, both for patients and dental personnel. Murdoch-Kinch 1997[7] listed four options to meet the ADA's proposed standard include independent water reservoirs, chemical water regimens, daily draining and purging regimens and point –of – filters. According to Charles M. Cobb[10] methods of reducing the numbers of colony forming units in DUWLs, include flushing the lines with water, intermittent or continuous use of bactericidal chemicals, radiation, self contained independent water reservoirs and filtration .Centers for Disease Control and Prevention recommended Infection Control practices for Dentistry 1993, urged the dentists to install and maintain anti-retraction valves to prevent the oral fluids from being drawn into DUWLs. They also recommended flushing waterlines daily for several minutes at

the beginning of the day and for 20 to 30 seconds between the patients to discharge oral fluids that may have entered the lines during the treatment. . Shannon Mills[54] in his review article in 2000 mentioned that the largest number of studies of waterline treatment published over the last 37years have investigated various chemical agents intended to inactivate microorganisms, induce detachment of biofilms or both. Chemicals can be introduced into the water systems either intermittently or continuously. According to Kettering [21] ADA goal can be achieved only when treated with distilled water and Chlorhexidine or Chlorhexidine alone. Chlorhexidine gluconate is a cationic bis biguanide group of antiseptic and disinfectant agent, which is active against various bacteria, viruses, bacterial spores and fungi. SE Mills[52] did experiment with undiluted Povidone-iodine 10%, loaded in five experimental units for 12 hours prevented recovery of microorganisms for 3 to 14 days when used in combination with sterile water reservoirs. Povidone iodine is formed by binding free iodine to polyvinyl-pyrrollidone and is a highly efficient microbicide to wide variety of bacterial, fungal and viral infections since 1960.

As a part of maintenance of aseptic field in dental clinic, it is important to know the bacterial load in dental unit waterlines before starting the treatment of dental units with chemical agents which is cost effective and a practically possible method to follow when compared to other procedures of disinfecting Dental unit waterlines. Hence my study was undertaken to evaluate the microorganisms present in the water samples collected from dental unit waterlines of different specialities which were randomly selected to find out the efficacy of two commonly available treating agents in disinfecting the dental unit waterlines.

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AIMS & OBJECTIVE

AIMS:

1. To enumerate and identify the microorganisms present in water samples collected from dental unit waterlines of different dental speciality clinics.
2. To find out the efficacy of two treating agents in disinfecting dental unit waterlines.

OBJECTIVE:

To achieve the recommendation issued by American Dental Association in the year 2000 that is "water for non surgical procedures should contain no more than 200 colony forming units /ml of aerobic bacteria in dental unit water" by treating the water with effective disinfecting agents.

REVIEW OF LITERATURE

The existence of contaminated water in dental units appears to have first been reported in 1963 in Great Britain by Dr. G.C. Blake.[5] Presence and identification of bacteria in the fluid from the 3 turbine handpiece supply reservoirs and 9 spray bottles filled with distilled water, tap water or tap water in which thymol mouthwash tablet dissolved were determined. After pipetting a 0.5ml sample of the contents into 15ml of glucose broth, a further 0.5ml poured into 15ml of Robertson's cooked meat media. After 24hours incubation at 37^{o}C, growth was subcultured onto blood agar and Mac Conkey plates for aerobic incubation at 37^{o}C. Further tests like sugar reactions, indole production, gelatin liquefaction, Voges Proskauer and methyl red reactions, citrate utilization and urease production were carried out for identification of microorganisms cultured. To assess the number of organisms present in the reservoirs, bacterial counts were made by pipetting 0.5ml of sample into 15ml of glucose broth. After thorough shaking, serial dilutions were immediately prepared and 0.1ml of this dilute fluid was spread on blood agar plates and incubated aerobically for 24 hrs at 37^{o}C and colonies were counted. The organisms such as Klebsiella aerogenes, Bacillus subtilis, and Pseudomonas pyocyanea were identified. The antibacterial effects of 1) chlorhexidine 2) thymol mouthwash solution 3) tap water at a temperature of 55 ^{0}C were then compared. After thorough cleaning 6 spray bottles were filled with 1:5000 solution of chlorhexidine gluconate in tap water and instructions were given to refill the bottles without getting emptied. After fourty- eight hours 0.5ml samples were taken from each

bottle into 15ml of chlorhexidine –neutralizing egg yolk medium and at the same time a small quality of contents was sprayed directly into 15ml bottles of egg yolk medium and incubated for 24hrs. Subculturing was done for the identification of organisms. He repeated the test using 1:10000 chlorhexidine. Samples were taken for seven days after filling the bottles and were repeated four weeks later and 3months later. Dr.G.C.Blake concluded that chlorhexidine in tap water at concentration of 1:5000 to 10000, was a satisfactory method of controlling infection both in bottles and in associated spray apparatus.

Shannon E. Mills, Patricia W. Lauderdale, Robert B. Mayhew (1986)[52] evaluated the reduction of microbial contamination in dental units with Povidone iodine 10%. The investigation was done in two phases. The first was a preliminary investigation designed to determine the most appropriate techniques for culturing and identifying the microbial flora present in dental unit waterlines. The second phase was the decontamination experiment. A total of 86 samples were assayed during the course of the preliminary investigations. In addition to 75 samples taken from dental unit water lines, ten samples were obtained from operatory sinks and one from a sterile water control. All culturing was done using plates that were incubated at either room temperature or at $35^{o}C$ .During the second phase ten dental units were surveyed for microbial contamination of the waterlines. All units were found to be colonized with bacteria and fungi at levels ranging from $9x10^{4}$ to $4.1x\ 10^{5}$ cfu/ml. Undiluted Povidone iodine 10% , loaded in five experimental units for 12 hours prevented the recovery of microorganisms for 3 to 14 days when used in combination with sterile water reservoirs.

Use of sterile water reservoirs alone did not effectively reduce the levels of microbial contamination in five control dental units.

M.V.Martin (1987)[39] described two case reports where medically compromised patients have been infected with Pseudomonas aeruginosa originating from the dental unit water supplies. A retrospective analysis of case records from the Liverpool Dental Hospital failed to show any infection in uncompromised patients that could be ascribed to contaminated Dental unit Water Systems. A prospective study of 9 dental units in which the Dental Unit Water Lines were contaminated with Pseudomonas aeruginosa showed that these microorganisms could be transferred to patients as a result of dental treatment, but failed to establish themselves as oral commensals to cause infection in non- medically compromised patients.

Shannon E. Mills, John C. Kuehne, Donald V. Bradley (1993) [53] done bacteriological analysis of high speed handpiece turbines. In 20 handpieces, no bacterial growth was found on any culture from an autoclaved or non autoclaved handpiece group. But growth occurred in the positive control inoculated with fresh whole human saliva. An adjunctive investigation with saliva substitute showed that oral fluids can contaminate handpiece turbine during simulated clinical treatment. Authors have given several possibilities to explain the failure to recover viable microorganisms which include lubricating oils or cleaners physically remove and or prevent the recovery of normal oral flora, physical action of turbine turning at speeds in excess of 300,000rpm in a

confined space, has a deleterious effect on bacterial viability or prevents adherence of organisms to the turbine assembly.

Henry N. Williams, Jacquiline Kelley, Doris Folineo, George C. Williams, Charles L.Hawley ,Joann Sibiski (1994)[18] assessed microbial contamination in clean water dental units and compliance with disinfection protocol. Twenty four clean water dental units surveyed were contaminated with large numbers of microorganisms. When units were properly disinfected and supplied with sterile water, they delivered water that was clean or not contaminated for at least 1week. They suggested that unsterilised tap water should not be used in dental units designed for the delivery of clean water and units that have been modified with a switching mechanisms to deliver water from the unit's bottle reservoir or from the community water supply should be carefully monitored and should be disinfected more often than weekly with 1:6 solution of household bleach and tap water.

Timothy F. Meiller, Louis G. Depaola, Jacqueline I.Kelley, Baqui,Been-Foo Turng, William Falkler (1999)[56] in their study ,excised tubing sections approximately 40cm in length from the end of air water syringe tubing on 11 dental units in the general dentistry clinics at Baltimore college of dental surgery and evaluated at baseline and after overnight treatment. Effluent water samples and biofilm samples from tubing sections also were evaluated by culture at baseline and after treatment with the chemical agents. Biofilm within the tubing was examined by Scanning Electron Microscopy and authors identified bacterial isolates using standard techniques. They also performed

minimum inhibitory concentration tests on identified isolates pre and post treatment and compared the results to determine possible differences in resistance. In baseline evaluations, effluent and biofilm matrix harboured an average of 1x 10^5 cfu/cm^2 and 1x 10^4 cfu/cm^2 recoverable microorganisms. A single overnight treatment of Dental Unit Waterlines with sodium hypochlorite, Gluteraldehyde or isopropanol 15.3% rendered samples free of recoverable bacteria and the level returned by the day six for Gluteraldehyde and day 15 for isopropanol. No evidence of resistance to agents was noted during the study.

Henry N.Williams, Marcie L. Baer ,Jacqueline I.Kelley (1995)[19]studied the contribution of biofilm bacteria to the contamination of the dental unit water supply as well as the effects of flushing and sodium hypochlorite treatment on reducing the number of contaminants. This study demonstrated that biofilm in the dental tubing was the primary source of contaminated water delivered by dental units and microbiologically clean water that was introduced into biofilm –laden tubing attached to a Clean Water dental unit became contaminated within 5 minutes.

Jean Barbeau(1996)[25] collected water samples from 123 dental units at the dental school of Universite de Montreal for bacterial identification and statistical analysis and found that none of the waterlines were spared from bacterial contamination. Thirty dental units out of 123 dental units were selected and 2-4ml water samples were collected directly from outlets of the polyethylene waterlines of the air/water syringe and high speed drill. Samples were collected at the beginning of the work day before the

dental unit was used and after a 2minutes purge corresponding to an average of 125ml of water and 300ml of air/water syringe. All the water samples were vigorously agitated with a vortex for 15 seconds. The plating was done by inoculating Petri dishes with 100µl of 1:10, 1:100 and 1:1000 dilutions in duplicate or by using an automatic spiral plating system, after a10 fold dilution of the sample. The enumeration was done with a magnifying glass and a counting grid. Newly installed dental units at the dental school were also sampled by the same sampling technique before their first clinical use. Sphyngomonas paucimobilis, Acinetobacter calcoaceticus, Methylobacterium mesophilicum and Pseudomonas aeruginosa were found as predominant isolates and significant differences in bacterial contamination between samples taken at the beginning of the day and samples taken after 2 min. purge were found. Difference were also found between water from the turbine and air/water syringe and in newly installed dental waterlines it was observed to take less than 5 days before initial bacterial counts reached a plateau of $2x10^5$ colony forming units /ml.

Carol Anne Murdoch-Kinch, Nancy L.Andrews, Salwa Atwan , Rick Jude , Michael J. Gleason , John A. Molinari (1997)[7] conducted a two month study to compare different quality management procedures in Dental Unit Water Lines using newly installed dental units. They evaluated independent water reservoirs, a sodium hypochlorite disinfection regimen, daily draining and purging of Dental Unit Water Lines and point of use filters by assessing microbial contamination and biofilm development using scanning electron microscopy. This investigation demonstrated that Dental Unit Water Lines contamination can be effectively controlled for prolonged periods by having new dental

units installed with non retracting water shut off valves, using distilled water in separate water supply.

James T.Walker, David J. Bradshaw,Allan M. Bennett, Martin R. Fulford, Michael V. Martin and Philip D. Marsh(2000)[23] investigated the microbial load of water from Dental Unit Water lines in 21 general dental practices and the biofouling of Dental Unit Water line tubing. Water and tube samples were taken from 55 dental surgeries in southwestern England. Contamination was determined by viable counts on environmentally selective, clinically selective, and pathogen-selective media, and biofouling was determined by using microscopic and image analysis techniques. Microbial loading ranged from 500 to 10^5 Colony Forming Units / ml in 95% of Dental Unit Water Line water samples, it exceeded European Union drinking water guidelines and in 83% it exceeded American Dental Association Dental Unit Water Line standards. Among visible bacteria, 68% were viable by BacLight staining, but only 5% of this "viable by BacLight" fraction produced colonies on agar plates. Legionella pneumophila, Mycobacterium species, Candida species, and Pseudomonas species were detected in one, five, two, and nine different surgeries, respectively. Presumptive oral streptococci and Fusobacterium species were detected in four and one surgeries, respectively, suggesting back siphonage and failure of antiretraction devices. Hepatitis B virus was never detected. Decontamination strategies significantly reduced biofilm coverage but significantly increased microbial numbers in the water phase .Microbial loads were not significantly different in Dental Unit Water Systems fed with soft, hard, deionized, or distilled water or in different Dental Unit Water Systems (main, tank, or

bottle fed). Microbiologically, no Dental Unit Water Systems can be considered "cleaner" than others.

M.Robert Wirthlin and Grayson W. Marshall (2001)[46] evaluated ultrasonic scaling unit waterline contamination after the use of Chlorine dioxide mouth rinse lavage. They compared the use of phosphate buffer- stabilized chlorine dioxide 0.1%mouthrinse in 15 scaler units with the use of tap water as a control. Sixteen ounce were run through the lines and allowed to sit undisturbed for 30 minutes, then flushed out with sterile water for 30seconds and the lines were purged dry with compressed air. Flushed out samples were cultured 7days at room temperature and colonies were counted. One test and one control unit were used for biopsy of internal tubing and scanning electron microscopy imaging which indicated a significant reduction of biofilm coverage by chlorine dioxide as compared to water.

Edward E.Putnins, Davide Di Giovanni and Amardeep S. Bhullar performed a study (2001)[14] on Dental unit waterline contamination and its possible implications during periodontal surgery. Approximately 40ml of water samples were obtained at random from 11 dental units in an established clinic. Scanning electron microscopy and bacterial viability staining were used to examine the sessile and planktonic biofilm present in dental unit waterlines and water samples and the limulus amebocyte assay was used to measure the lipopolysaccharide levels in water samples. Viability staining technique identified significantly more bacteria and 64% of total bacterial population stained as non vital. The mean Lipopolysaccharide levels in water collected from high

speed and air/waterlines in use were 480 and 1,008 endotoxin units. The Lipopolysaccharide levels at the start of the day were reduced by 70% with 1minute of flushing .Flushing times of 5and 10minutes were not able to reduce Lipopolysaccharide levels to zero.

Jackson B. Linger , John A .Molinari , William C. Forbes conducted a study (2001)[20] to investigate the use of a hydrogen peroxide- based dental unit waterline treatment to reduce the colonization and growth of heterotrophic bacteria. Twenty- three dental units with self contained water systems were randomly selected in which three units and tap water served as controls. Twenty four water samples were taken at baseline and once a week for 5 consecutive weeks. They were serially diluted, spread plated in duplicate onto R2A agar plates and incubated at 37^0C for 7days.Results showed at baseline, tap water control with mean count of 0 cfu/ml, three control Dental Unit Water Lines with a mean count of 8,440 cfu/ml and the twenty treated Dental Unit Water Lines had counts of less than 200cfu/ml and by week 4, median count for all of the treated Dental Unit Water Lines was 0cfu/ml.

Jean Barbeau, Tania Buhler (2001)[26] carried out a study on the biofilms to detect, observe and evaluate the concentration of free-living amoebae in dental unit waterlines. Fifty-three water samples were collected from 35 dental units (air/water syringes) and 18 water taps. The technique was based on the ability of waterborne bacteria to create a biofilm and serve as substratum for the development of amoebae naturally present in the water samples. Laboratory grown fresh water biofilms support the proliferation of a wide variety of free living amoebae. All the dental unit water samples tested contained

amoebae at concentrations upto 330/ml or more than 300 times the concentration in tap water from the same source. Hartmanella, Vanella, Vahlkampfia species were the most frequently encountered. Naegleria and Acanthamoeba species were also present in 40% of the samples.

James D.Kettering ,Joni A. Stephens, CarolisA Munoz-Viveros ,W.Patrick Naylor (2002)[21] in their study evaluated microbial contamination in water samples from 75 new dental units with a closed circuit water system and were compared using combinations of tap water and sterile distilled water with and without two chemical disinfectants (bleach and 0.12 % chlorhexidine gluconate) over a six week period. Baseline tap water were collected and tested initially and microbial counts in these specimens were ranged from 4-95 cfu/ml. When passed through dental units, no significant bacterial reduction was achieved for the samples of tap water, tap water treated with Bio 2000. Only water samples from dental units using Bio2000 alone or a combination of sterile, distilled water with Bio 2000 met the 200cfu/ml standard goal for dental treatment water.

Lucio L Montebugnoli and Giovanni conducted a study (2002)[36] to evaluate the efficacy of a new chemical solution flushed through Dental unit waterlines for the control of contamination inside dental units. Water samples were collected from six old dental units equipped with a device designed to automatically flush disinfecting solutions through the water systems, before and after 5minutes Dental Unit Water Lines disinfecting cycle with Tetra acetyl Ethylene Diamine (TAED) and persalt. They found that there was significant difference in Dental unit water line contamination with respect

to baseline when the water samples were tested in vitro and after undergoing a 5minutes disinfecting cycle with the chemical agent.

Charles Cobb, Christopher R. Martel, Sidney A. McKnight, Cathy Pasley-Mowry, Brett L. Ferguson, Karen Williams (2002)[10] conducted a study to evaluate how time dependent waterline flushing affects the presence of biofilms in other wise untreated dental unit waterlines. 50ml of baseline water samples were collected from 12 handpiece lines prior to the start of continuous flushing. Additional 50ml were collected after two, three, four minutes flushing intervals from the baseline. Levels of planktonic bacteria in Dental Unit Water Lines were quantified by counting colony forming units. Segments of water tubing from each of high speed handpiece waterlines were examined by Scanning Electron Microscopy, which confirmed the presence of a residual biofilm in the lumen of each dental unit waterlines. In this study Cobb et al concluded that water flushing of Dental Unit Water Lines produced a significant reduction in planktonic bacteria at each time interval compared to baseline and between each successive time intervals and the level of colony forming units/ml after 4minutes of flushing still exceeds the current American Dental Association recommendations for acceptable level of microorganisms.

M.C.M. de Souza –Gugelmin (2003)[37] performed a study to evaluate the occurrence of microbial contamination in dental unit waterlines. Water samples were collected aseptically from the waterlines (reservoir, triple syringe, high speed equipment) of 15 dental units. After serial dilutions to $1:10^6$ in APHA (eluent provided by American

Public Health Association, Composition: 34gm monobasic phosphate and 100ml distilled water) and the samples were seeded by the pour plate technique. Incubated for 48hrs at 32^0C in plate count agar. The number of colony forming units was determined in each plate after incubation and cfu/ml was calculated by number of colonies x dilution factor. Results showed that the levels of contamination were highest in the triple syringe and in high speed equipment and no significant statistical differences between the level of contamination in the triple syringe and high speed equipment found.

Nuala B. Porteous (2003)[42]conducted a study to test the efficacy of a continuous use, stabilized Chlorine dioxide proprietary compound to decrease the number of bacteria in Dental Unit Water Lines. They used three dental units with self contained water systems to test the product and three similar units as controls. They aseptically collected water samples weekly according to recommended methods, plated the samples on R2A agar and incubated them for 7 days. The authors isolated heterotrophic, mesophilic bacteria from treatment and control units for 8 weeks. In the 9^{th} week, predominant isolates from one of the treatment units changed in appearance to small, dark, shiny colonies and identified as fungi Exophiala mesophila. Similar colonies were also isolated from source tap water and ultra sonic and handpiece lines. This was not observed in control units. So they concluded that continuous waterline treatment might alter the natural water flora and promote the growth of a fungus which was already present in small amounts in municipal water supply.

M. Robert Wirthlin, Grayson M. Marshall, Randal W. Roland (2003)[47] compared three dental unit waterline cleaners (an alkaline peroxide product, freshly mixed chlorine dioxide product, buffer stabilized chlorine dioxide product) in 16 dental units with self contained water system , after 6months of installation in a periodontal teaching clinic. One unit treated by flushing and drying is used as a control. Units were sampled daily for 10 days with heterotrophic plate count (HPC) sampler plates and incubated for 7 days at room temperature and colonies were counted .Samples from internal water tubing before and after the use of waterline cleaners were processed and examined by scanning electron microscopy. Results showed, freshly mixed chlorine dioxide and buffer stabilized chlorine dioxide both reduced Heterotrophic Plate Count to near 0 in all days. When comparing the two the buffered chlorine dioxide was better than alkaline peroxide at all times. Scanning electron microscopy showed reduction in biofilm coverage, but the differences before and after was not statistically significant.

Ruby Singh, O. Colin Stine,David L . Smith, John K. Spitznagel Jr., Mohamed E. Labib, and Henry N. Williams (2003) [49] investigated the microbial diversity of biofilms found in dental unit water systems (DUWS) by three methods. The first was microscopic examination by scanning electron microscopy (SEM), acridine orange staining, and fluorescent in situ hybridization (FISH). Most bacteria present in the biofilm were viable. Fluorescent In Situ Hybridization detected the ß and [illegible], but not the [illegible], subclasses of Proteobacteria. In the second method, 55 cultivated biofilm isolates were identified with the Biolog system, fatty acid analysis, and 16S ribosomal DNA (rDNA) sequencing. Only 16S identified all 55 isolates, which represented 13 genera. The most common organisms belonged to the genera Afipia (28%) and Sphingomonas (16%).The

third method was a culture-independent direct amplification and sequencing of 165 subclones from community biofilm 16S rDNA . This method revealed 40 genera: the most common ones included Leptospira (20%), Sphingomonas (14%), Bacillus (7%), Escherichia (6%), Geobacter (5%), and Pseudomonas (5%). Their results have demonstrated that a biofilm in a health care setting might harbor a vast diversity of organisms. The results also reflect the limitations of culture-based techniques to detect and identify bacteria. Although this is the greatest diversity reported in Dental Unit Water System biofilms, other genera might have been missed. Using a technique based on jackknife subsampling, they projected that a 25-fold increase in the number of subclones sequenced would approximately double the number of genera observed, reflecting the richness and high diversity of microbial communities in these biofilms.

J. T. Walker, D. J. Bradshaw, M. R. Fulford, and P. D. Marsh (2003)[59] conducted a study regarding the Microbiological Evaluation of a Range of Disinfectant Products to Control Mixed-Species Biofilm Contamination in a Laboratory Model of a Dental Unit Water System. The aim of this study was to use an established biofilm laboratory model to simulate biofouling of Dental Unit Water Systems to evaluate practical, cost-effective, and evidence-based methods of microbial decontamination. Reproducible biofilms were developed in the model over 14 days; decontamination was assessed using total viable counts (TVC) and microscopic-image analysis techniques to view the inner surface of tubing. Flushing did not reduce the biofilm coverage or Total Viable Counts. Combizyme and ozone did not completely eliminate the viable bacteria (70 and 65% reduction in biofilm TVC, respectively), nor did they remove the biofilm (45 and

57% reduction in biofilm coverage, respectively). Chlorhexidine and Bio2000 (active agent: ethanol and chlorhexidine), Tegodor and Gigasept Rapid (aldehyde based), and Grotanol (hydroxide based) completely eliminated the Total Viable Counts but did not completely remove biofilm (31, 53 33, 34, and 64.9% reduction of biofilm coverage, respectively). Other products including Grotanol Flussig (phenol based), Betadine (povidone-iodine based), Alpron (chlorite based), and the hydroxide-containing products Sporklenz, Sterilex Ultra, Dialox, Sterilox, Sanosil, Oxigenal, and Grotanat Bohrerbad resulted in a 100% reduction in the biofilm Total Viable Count and a >95% reduction in biofilm coverage. The study demonstrated that while many disinfectants achieve a sufficient reduction in Total Viable Count they might not necessarily remove unwanted biofilm from the tubing surfaces as tested in this laboratory-controlled biofilm model.

Nuala B.Porteous (2004) [43] performed a study to test the efficacy of an intermittent use,dental unit waterline cleaner containing 0.12% chlorhexidine to reduce the bacterial levels in three functional units with independent water reservoir systems. In this study first baseline water samples were taken from six units. In three units two ounces of the undiluted test product was run through lines, left overnight and flushed out next morning. This was repeated for six nights initially and once a week thereafter for 12 weeks. Weekly samples were collected in bottles containing sodium thiosulphate on the afternoon before overnight treatment, plated on R2A agar and incubated at room temperature for 7 days and could found that intermittent treatment of dental units with 0.12% chlorhexidine gluconate resulted in significant reduction of bacterial counts to

levels that were consistently below American dental association's goal of 200cfu/ml for 8 weeks.

James W. McDowell, Daryl S. Paulson, John A. Mitchell (2004)[24] conducted a study on a strategy for preventing biofilm formation in10 dental unit waterlines. The authors used a simulated use dental unit waterline system to evaluate the ability of a test product, A-dec ICX (A-dec, Newburg, Ore.), to prevent biofilm formation. They evaluated buffered distilled water and hard water models versus mixed challenge suspensions of Staphylococcus aureus, Klebsiella pneumoniae and Pseudomonas aeruginosa. Results showed the presence of a bacterial challenge of 100 to1000cfu/ml in the incoming water and A-dec ICX effectively prevented the development of biofilm and maintained water quality at a level consistently well below 200cfu/ml at both high and low water hardness levels .

Vijay Venkatesh, Vidyashree Nandhini, Velmurugan, Dr. Parameswaran, Dr. Kandaswamy (2004)[58] evaluated the bacterial contamination of dental unit waterlines and the efficacy of a commercial disinfectant (Sterilex Ultra) in eliminating biofilms from dental unit waterlines. After collecting random water samples from water booster, air turbine, air water syringe of three dental units for bacteriological analysis, a commercially available disinfectant, Sterilex Ultra was used to treat dental waterlines. Water samples from different parts of dental unit waterlines were collected on 3rd , 5th, 7th day following treatment with the reagent and the samples were sent for bacteriological examination. One inch tubing from the outlet of the booster, air turbine

and air/ water syringe was sectioned and processed for bacteriological examination. In this study Vijay Venkatesh concluded that disinfectant use was found to be effective for a period of 6 days and for maintenance of sterility of dental unit waterlines a good source of water, an effective disinfectant, use of an antiretraction valve is essential.

Toshiak Yabuna Japan (2004)[57] compared newly installed dental units that were equipped with either a conventional polyurethane tube (unit A) or a polyvinylidene fluoride (PVDF) tube (unit B), and the numbers of bacteria discharged from high- and low-speed handpiece lines were counted using R2A agar plates. Bacterial attachments on surfaces were observed with a scanning electron microscope up to 185 days. Bacterial outflow during 1-day clinical service from a Dental Unit Water Lines after 1-year usage was also examined. The surface free energy of each tube was determined based on the measurement of contact angles. The number of bacteria discharged from unit B was lower than from unit A at 80 days and thereafter. Scanning Electron Microscopic examination demonstrated that the unit A tube was covered by biofilm constituting rods and filaments after 94 days, while no biofilm was observed in the unit B tube even after 185 days. After 1-year of usage, the unit B released significantly less bacteria than the unit A at every sampling period of 1-day clinic work. Surface free energies, calculated from contact angles measured, of Polyvinylidene fluoride and polyurethane tubes were 37.7 and 77.8, respectively. He concluded that the Polyvinylidene fluoride tubes, which have lower surface free energy than the conventional tubes, were effective in inhibiting biofilm formation and reducing bacterial outflow from Dental Unit Water Lines.

Szymanska J, Wdowiak, Puacz E, Stojck NM (2004)[28] conducted a study to assess microbiologically the water contained in the dental unit water. Water samples were collected aseptically from the water reservoirs of 19 dental units. Results showed that 63.1% of samples, the number of colony forming units cfu/ml and of coliform organism significantly exceeded acceptable values.

Chate[11] conducted a study between 2002 to 2004 to improve the quality of water emanating from dental unit waterlines. Samples of water collected from Dental Unit Water Lines of three geographically separate district dental facilities of United Kingdom NHS trust, prior to the start and midway through a morning session. These were tested microbiologically within six hours of sampling. One of the clinics followed flushing of water through its Dental Unit Water Lines while other two clinics used intermittent disinfection purging regimens, one with two stage protocol of Ethylene Diamine Tetra acetic acid followed by hydrogen peroxide and other with Bio 2000 as a single agent. Dental units using Bio2000, colony counts remained below the European union recommendation level.

Frank A. Scannapieco, Alex W. Ho, Maris DiTolla,Casey Chen, Andrew R. Dentino (2004)[17] conducted a study to determine the prevalence of respiratory disease among dental students with their exposure to the clinical dental environment. A detailed questionnaire was administered to 817 students at three dental schools. The questionnaire sought information concerning demographic characteristics, school year,

exposure to the dental environment and dental procedures, and history of respiratory disease. Respondents reported experiencing the following respiratory conditions during the previous year: asthma (26 cases), bronchitis (11 cases), chronic lung disease (6 cases), pneumonia (5 cases) and streptococcal pharyngitis (50 cases). Statistical analyses indicated no significant associations between the prevalence of any of the respiratory conditions and year in dental school, except for asthma, for which there was a significantly higher prevalence at 1 school compared to the other 2 schools. When all cases of respiratory disease were combined as a composite variable and subjected to multivariate logistic regression analysis controlling for age, sex, race, dental school, smoking history and alcohol consumption, no statistically significant association was observed between respiratory condition and year in dental school or exposure to the dental environment as a dental patient. The authors concluded that no association was found between the prevalence of respiratory disease and a student's year in dental school or previous exposure to the dental environment as a patient. These results suggest that exposure to the dental environment does not increase the risk for respiratory infection in healthy dental health care workers.

A study conducted by Samir E. Bishara, Manal Soliman, Raed Ajlouni (2005)[50] to determine the use of an iodine compound for disinfecting waterlines in dental units and its effect on shear bond strength of orthodontic brackets bonded to enamel. Fourty extracted molar teeth were collected and stored in a solution of 0.1% w/v thymol. Twenty teeth in group I control were etched for 15 seconds with 35% phosphoric acid, washed with a distilled water spray for 10seconds, stored in distilled water for five

minutes, then dried and sealant applied to etched surface. Other twenty teeth in group II experimental were etched for 15 seconds with 35% phosphoric acid and washed for 10 seconds with water containing iodine and stored in iodinated water for 5minutes, dried and sealant applied to the etched surface. Pre coated brackets were placed on all the teeth and light cured for 20 seconds. All teeth were debonded within thirty minutes from the initial time of bonding and no significant difference was found in the shear bond strengths of the teeth that were washed and immersed in iodine solution and the control group in which distilled water was used.

Schel, Marsh, Bradshaw (2006)[51] conducted a study comparing disinfection products for their ability to meet American Dental Association's guidelines of <200cfu/ml for Dental Unit Water System water. Alpron ,Bioblue ,Dentosept, Oxygenal, Sanosil, Sterilex Ultra, Ster 4 spray were tested in 134 Dental Unit Water lines in Denmark, Germany, Greece, Ireland, Netherlands, Spain and United Kingdom. Weekly water samples were tested for viable counts on yeast extract agar upto 4-5 week at baseline followed by 6-8 weeks of disinfection. They found that most effective products were Dentosepy and Oxygenal where Dentosept gave the most consistent and sustained antimicrobial effect over time and continuously applied products performed better than those applied intermittently.

Jolanta Szymanska (2006)[30] did a bacteriological assessment of the dental unit waterlines (DUWL) biofilm - concentration and composition of the aerobe and facultative anaerobe bacterial microflora, and evaluation of the influence of a

disinfecting product, Oxygenal 6, on the biofilm composition. Tubing fragments were taken from 25 units twice, before and after disinfection, and bacterial suspension of the biofilm was obtained from the samples. The bacterial flora was determined with the plate culture method. Bacteria were identified with biochemical microtests: API 20E, API 20NE (bioMérieux, France) and GP2 MicroPlateTM (BIOLOG, USA). Before disinfection, the following bacteria were identified: Gram-negative bacteria - Ralstonia pickettii, Pseudomonas vesicularis, Sphingomonas paucimobilis, Xanthomonas maltophilia; Gram-positive cocci -Micrococcus luteus, Micrococcus lylae, Staphylococcus cohnii, Staphylococcus lentus, Staphylococcus species, Streptococcus species; Actinomycetes - Streptomyces albus. The prevailing bacteria were: Ralstonia pickettii (78.62%), found in all the units, and Sphingomonas paucimobilis (20.45%). After Dental Unit Water Lines disinfection, Sphingomonas paucimobilis (88.79%) dominated in the biofilm, Staphylococcus species. - 5.61% and Pseudomonas species. - 3.74% was the next most frequently occurring bacteria, and in more than a half of the biofilm samples 100% reduction of the bacterial microflora occurred. This study confirmed the effectiveness of Oxygenal 6 in bacterial decontamination of the Dental Unit Water Lines biofilm.

Jolanta Szymanska (2006)[31] conducted a study regarding the mycological assessment of bioaerosol forming during conservative dental treatment, taking into account concentration and type of fungal microflora, and evaluation of the influence of Dental Unit Water Lines disinfecting protocol on the fungal contamination of the bioaerosol. The research was conducted on 25 operative sites located in public dental clinics. The

air contained in the space between a patient and a dentist during conservative dental treatment with the use of a high-speed handpiece was examined. Air samples were taken using the portable RCS PLUS Air Sampler (BIOTEST AG, Dreieich, Germany) and ready-to-use agar YM Strips for yeast and mould fungi culture. The volume of the sampled air was 100 litres. Before disinfection, the concentration of fungi in the collected air samples at individual operative sites ranged from 4×10^{1}cfu/m^3 to 34×10^{1}cfu/m^3. The most common species was Penicillium herquei (62.17% of the total count), followed by other fungi: Alternaria alternata - 12.68%, Penicillium roseopurpureum - 9.41%, Rhizopus nigricans - 5.93%, Aspergillus terreus - 3.89%, Geotrichum candidum - 2.25%, Aspergillus glaucus group - 2.04%, Cladosporium cladosporoides - 1.23% and Penicillium diversum - 0.41%. The concentration of Penicillium herquei at individual operative sites ranged from 0 to 34 × 101 cfu/m^3, mean 121.6 cfu/m^3, Penicillium roseopurpureum -from 0 to 11×10^{1} cfu/m^3, mean 18.4 cfu/m^3 and Alternaria alternata - from 0 to 18×10^{1} cfu/m^3, mean 24.8 cfu/m^3. After disinfection, like before disinfection procedures,the prevailing species of fungi were: Penicillium herquei, Penicillium reseopurpureum and Alternaria alternata, which amounted to 62.6%, 18.28% and 11.36% of the isolated fungi, respectively. The recorded levels of total airborne fungi were lower after Dental Unit Water Lines disinfection compared to those before disinfection.

Mark E. Stone, Johnc. Kuehne (2006)[38] had undertaken a study to determine whether iodine used to control bacteria in dental unit waterlines could increase mercury concentrations in dental wastewater. The study was conducted in four parts. Part1.

Solutions containing iodine in concentrations ranging from zero (control) to 20 mg/L were mixed with ground and sieved dental amalgam and then allowed to equilibrate by settling. Cold vapor atomic absorption spectrometry was used to determine mercury levels in the settled supernatants at 24 hours and at 7 days. Part 2. Deionized water was pumped through an iodine-releasing water-treatment cartridge, collected, and mixed with ground and sieved dental amalgam. Mercury levels in settled supernatants were measured at 24 hours and at 7 days. Part 3. Iodine in water from two commercial iodine-releasing cartridges was measured using Inductively Couple Plasma Mass Spectrometry. Part 4. Baseline mercury levels in settled supernatants from wastewater collected from two dental chairs were compared to samples taken from chairs equipped with iodine-releasing cartridges. Authors found that iodine, used to control biofilm and bacteria in dental unit waterlines, can mobilize mercury from amalgam particulate in dental unit waste water, resulting in higher levels of mercury in waste water. They concluded that practices using amalgam as a dental restorative material should consider alternatives to halogen containing products to control biofilm and bacteria in water used for dental procedures.

Jolanta Szymanska (2007)[32]conducted a study to evaluate the bacteriological assessment of water in dental unit reservoirs, concentration and composition of the aerobic and facultative anaerobe bacterial microflora. Reservoir water samples were taken from 25 dental units. Bacterial flora was determined with plate culture method. Bacteria were identified with biochemical microtests and concentration of bacteria isolated ranged from 22,300cfu/ml to 5,83,000cfu/ml. Szymanska J concluded that as

the bacterial concentration in dental unit reservoirs reached the excessive values and composed of bacteria characteristic for water supply systems, opportunistic pathogens and bacteria of oral cavity flora , continuous microbiological monitoring of Dental Unit Water Lines water including application of a disinfecting procedure is necessary.

André V. Ritter, Eduardo Ghaname, Ralph H. Leonard (2007) [4]checked the influence of dental unit waterline cleaners on composite-to-dentin bond strengths. The authors tested the strength of resin-based composite bonded to dentin in specimens treated with distilled water (control) or one of four cleaners. Cleaners used were Sterilox, ICX, Sodium hypochlorite and MicroClear, The authors randomly divided 150 caries free human premolars into 5 groups of 30 specimens. They tested a total-etch adhesive, a self-etching primer/adhesive and an experimental self-etching primer/adhesive. The specimens were stored for 24 hours at 37^0 C and then tested them to determine their bond strengths. They concluded that bonding of resin based composites to dentin is not affected by the cleaners tested.

AS Al-Hiyasat , SY Ma'ayeh, MY Hindiyeh, YS Khader (2007)[2] evaluated the extent of Pseudomonas aeruginosa contamination of dental unit water at a teaching center in Jordan. Water samples were collected from 30 dental units, 10 from each of three teaching clinics, namely Conservative dentistry, Periodontology and Prosthodontics. Samples were collected from the outlet of air /water syringe , high speed handpiece and water cup filler, at the beginning of the working day (before use) , after 2minutes flushing and at mid day. Pseudomonas aeruginosa was detected in 86.7% of the dental

units at the beginning of the working day and in 73.3% after 2minutes of flushing and at midday.Conservative dentistry units had the highest counts, followed by Periodontology and Prosthodontics .Overall highest counts were at the beginning of the day and the lowest counts after flushing for 2 minutes and highest numbers were seen again at midday, thus showing that flushing the Dental Unit Water for 2minutes significantly reduced the counts of Pseudomonas aeruginosa.

METHODOLOGY

The present study was conducted in the department of Pedodontics & Preventive dentistry, Ragas Dental College to assess the biofilm of Dental unit waterlines from various speciality dental clinics and to check the effect of treating agent used to disinfect the Dental unit waterline. Sample included 70 dental unit waterlines from different speciality dental clinics which were checked for microbial contamination. From these dental units 40 units were randomly selected and divided into two groups of 20 each. Group A, (20 dental units) was treated with 0.2% Chlorhexidine gluconate solution and Group B, (20 dental units) was treated with 10% Povidone iodine solution and the reduction in the microbial levels were assessed. Five dental units were randomly selected and checked for the microbial contamination using mineral water, sterile distilled water, fresh tap water as a water source in the dental unit reservoir bottles. Five dental units were randomly selected to collect water samples from three different water outlets such as handpiece lines, air/water syringe and scaler lines and microbial contamination was assessed. The duration of efficacy of treating agent was checked in 5 samples from each group for one week at 3, 5 and 7day intervals.

Inclusion criteria:[36]

- Units that had been in daily use for approximately one year.
- Units that have not been treated for removal of biofilm or reduction of planktonic bacteria.

Sample collection at baseline

Water samples were collected from the end of operator's water syringe line of 70 dental units from different specialities using sterile techniques.[10] (The sterile techniques include the use of sterile gloves, wiping the external surfaces of water line with sterile cotton gauze soaked in 70% alcohol and collection of waterline samples in sterile bottles). Before sample collection the reservoir bottle on each unit was washed and disinfected, then filled with fresh tap water and reattached to the dental unit. Fresh tap water was collected in a bottle before sample collection to assess the microbial levels for baseline evaluation. Lines were flushed for 20 seconds if dental unit was in use that day or for 2minutes if the unit was not in use.20ml water was collected from water syringe line in a sterile bottle. Water splashing was minimized when filling the container and any contact between air/water syringe and the container was avoided. The samples were transported immediately to the laboratory for microbial evaluation.

In a similar manner, water collected from different outlets (Handpiece lines, Air water syringe lines, Scaler lines) and from different source (Mineral water, Sterile distilled water, Fresh tap water) were analyzed in randomly selected 5 samples.

Laboratory procedure

Ten fold dilutions of each unit sample were made in sterile phosphate buffer solution. Phosphate buffer solution was prepared by mixing 19ml of Na2HPO4. 2 H2O, 0.2M Solution with 81ml of , NAH2PO4.H2O,0.2 M solution and diluted to a total of 200ml at the pH 7.4.Autoclaving was done to get the sterile solution .1/10 dilution was made by mixing 1ml of sample with 9ml of sterile phosphate buffer solution.

Samples were vigorously agitated by vortex for 15 seconds. 0.1ml of one tenth milliliter of each dilution was plated on R2A agar using spread plate method and kept in the incubator at 35^0 C for 5 days.

Composition of R2A agar

Enzymatic digest of Casein ----------0.25gm

Enzymatic Digest of Animal tissue---0.25gm

Acid Hydrolysate of Casein ---------- 0.5gm

Yeast Extract----------0.5 gm

Dextrose----------0.5gm

Soluble starch----------0.5gm

Dipotassium phosphate----------0.3gm

Magnesium sulfate Heptahydrate-----0.1gm

Sodium Pyruvate----------0.3gm

Agar--- ----------15gms

Final pH: 7.2+_0.2 at 25^0 C

Preparation of medium

18.2 gms of R2A agar was suspended in one liter distilled water and heated in a flowing steam until the medium completely dissolved. It was then autoclaved for 15min., at 121^0 C, cooled to 45-50 ^{0}C and poured onto sterile Petri dishes. The prepared medium was clear to slightly opalescent and colorless. Plates were protected from light and dehydration and stored in the refrigerator.

Enumeration was done with the help of magnifying glass[25] by counting the total colony forming units irrespective of the type and genera. Each colony was assessed for the

identification of the microorganisms and confirmed by using Gram Staining[32] and biochemical tests.[2]

Gram stain was used in identification of bacteria which helps differentiate Gram positive organisms and Gram negative organisms.[3]

Oxidase Strips were used to detect the presence of the enzyme Cytochrome Oxidase produced by a number of bacteria such as Pseudomonas, Neisseria and Campylobacter. Positive result was indicated within a few seconds by smeared area turning deep purple.

Triple sugar Iron agar slant was used to confirm the presence of the bacterias such as E.Coli, Pseudomonas, Proteus based on their sugar fermenting capacity. Two or three colonies of test organism on agar medium were touched by using a loop, inoculated onto the agar slants. Identification of bacterias was done based on the color changes and gas production that was detected within 18-24hrs.[3]

Treatment with 0.2% Chlorhexidine &10% Povidone Iodine

The self contained reservoir bottle were filled with 25ml of treating agent either 0.2% Chlorhexidine or 10% Povidone iodine solution and run through the waterlines for 30 seconds and left in the lines overnight. The following morning, self contained reservoir bottle was removed and filled with fresh tap water and the product was flushed out until clear water could be seen. Water samples were collected from water syringe lines and microbiological analysis was done by following the same procedure as the baseline.

Sample collection was done in 5 units on the 3rd, 5th, 7th day after treating the lines with the disinfectants to check the duration as well as efficacy of the treating agent.

RESULTS

The study was conducted by the department of Pedodontics and Preventive dentistry, Ragas Dental College to assess the biofilm of Dental Unit Waterlines from various speciality dental clinics and to check the effect of treating agent used in disinfecting the dental unit waterlines. Samples included 71 dental unit waterlines from different speciality dental clinics which were checked for microbial contamination .From these dental units, 40 dental units were randomly selected and divided into two groups of each.

Group A, was treated with 0.2% Chlorhexidine & Group B was treated with 10% Povidone –Iodine and reduction in microbial contamination were checked.

Table 1-9 shows the study results

Table I- Baseline contamination & Prevalence of microflora

Sample No	Baseline cfu/ml	*Microorganism isolated*								
		Pseudomonas	*Candida*	*M.M.*	*E.Coli*	*Bacillus*	*Klebsiella*	*Proteus*	*Serratia*	*Staphylo-cocci*
1	22200	+								+
2	17000	+								+
3	31600	+	+				+			+
4	8700	+					+			+
5	21400	+								+
6	4400	+								+

7	3200	+		+					+	+
8	12400	+				+				+
9	11200	+								+
10	17800	+	+			+	+		+	+
11	5600	+								+
12	13600	+				+	+		+	+
13	6700	+								+
14	15200	+								+
15	8400	+	+							+
16	22000	+								+
17	29200	+	+				+			+
18	19600	+	+			+		+		+
19	11200	+								+
20	27600	+	+				+		+	+
21	9400	+	+				+			+
22	2800	+	+			+	+			+
23	28600	+				+	+	+		+
24	12400	+								+
25	9800	+								
26	6500	+		+						
27	15400	+				+	+		+	+
28	4500	+								+
29	16300					+				+
30	6800	+	+			+	+			+
31	8700	+				+				+
32	10800	+			+					+
33	34200	+			+	+		+	+	+

34	28900	+			+		+			
35	11600	+	+	+			+			+
36	22000	+				+			+	+
37	56800	+	+		+	+				+
38	40800	+	+			+	+			+
39	4800	+	+	+		+		+	+	+
40	7400	+	+					+	+	+
41	32600	+								+
42	7300	+								+
43	22400	+								+
44	12300	+	+				+		+	+
45	6100	+								+
46	13600	+					+			+
47	19200	+						+		
48	13700	+	+						+	+
49	11100	+								+
50	10100	+	+						+	+
51	10400	+								+
52	12400	+								+
53	10100	+				+		+		
54	7200	+							+	+
55	44800	+	+			+		+		+
56	13800	+				+	+		+	+
57	18400	+					+		+	+
58	34000	+	+			+	+		+	+
59	30800	+	+			+	+		+	+
60	7800	+				+				+

61	68000	+					+			+
62	16600	+	+				+		+	+
63	5500	+	+				+		+	+
64	50000	+						+		
65	6400	+	+			+	+			+
66	25600	+	+			+		+	+	+
67	19800	+	+	+			+		+	+
68	21400	+				+		+	+	+
69	10300	+						+		+
70	27800	+						+		+
71	*16800*	+					+	+		+
		70	25	5	4	24	26	14	22	65
		(98.59%)	**(35.21%)**	**(7.04%)**	**(5.63%)**	**(33.8%)**	**(36.62%)**	**(19.72%)**	**(30.99%)**	**(91.55%)**

Using Chi-Square test P value is found to be <.001

**** denotes significant at 1%level**

Min. value ---2800 cfu/ml

Max. value ---68000 cfu/ml

Range---------- $2.8x10^3$ to $6.8x\ 10^4$ cfu/ml

Mean value—18380.28 cfu/ml ($1.8\ x10^4$ cfu/ml)

Table I represents the bacterial profile of the water samples collected from the 71 dental unit waterlines. The cultures from water samples showed the presence of following microorganisms in the order of descending frequency- Pseudomonas 70 (98.59%), Staphylococci 65 (91.55%) , Klebsiella 26(36.62%) , Candida 25 (35.21%), Bacillus 24(33.8%), Serratia22 (30.99%), Proteus14 (19.72%), Methylobacterium

Mesophilicum 5 (7.04%) ,E. Coli 4 (5.63%) . The colony count varied from 2800cfu/ml to a maximum of 68000cfu/ml with a mean colony forming units of 18380.28/ml. The variation is statistically significant (P value<.001).

Table II

Baseline values & reduction after treatment with 0.2% Chlorhexidine

Treating agent	No. of dental units	Pretreatment values	Post treatment	% reduction	P value
0.2% Chlorhexidine	1	22800	1700	92.54%	**<.001****
	2	7500	2400	68%	
	3	22800	6600	71.05%	
	4	24000	2000	91.66%	
	5	42800	3400	92.05%	
	6	6800	1900	72.05%	
	7	67000	6200	90.74%	
	8	23600	2500	89.40%	
	9	45400	4500	90.08%	
	10	28600	2600	90.90%	
	11	62400	5400	91.34%	
	12	14400	3800	73.61%	
	13	14200	2100	85.21%	
	14	13800	2500	81.88%	
	15	11000	1200	89.09%	
	16	16000	1800	88.75%	
	17	14000	1000	92.85%	
	18	8300	800	90.36%	
	19	6500	400	93.84%	

	20	9600	1100	88.54%	
Mean +-S.D.	**20**	**23075+-17912.47**	**2695+-1773.63**	**86.2%**	

Table II shows the efficacy of 0.2% Chlorhexidine in reducing the microbial contamination in 20 dental units. Mean baseline contamination was found to be reduced by 86.2% after treatment. This percentage reduction was observed to be statistically significant, when analyzed using Paired T tests followed by Wilcoxon Signed Rank tests

Table III

Baseline values & reduction after treatment with 10% Povidone iodine

Treating agent	No. of dental units	Pretreatment values	Post treatment	% reduction	P value
10%Povidone Iodine	1	4500	0	100%	**<.001****
	2	5400	200	96.29%	
	3	12600	500	96.03%	
	4	5800	300	94.82%	
	5	5600	500	91.07%	
	6	5700	0	100%	
	7	3400	0	100%	
	8	6900	100	98.55%	
	9	6100	1000	83.60%	
	10	7400	900	87.83%	
	11	16300	500	96.93%	
	12	3300	0	100%	
	13	6800	100	98.52%	
	14	8700	200	97.70%	

	15	14000	300	97.85%	
	16	14000	400	97.14%	
	17	3200	100	96.87%	
	18	4400	200	95.83%	
	19	2800	100	96.42%	
	20	5600	100	98.21%	
Mean +_S.D.	**20**	**7125+-3978.88**	**275+_286.31**	**96.14%**	

Table III shows the efficacy of 10% Povidone iodine in reducing the microbial contamination in 20 dental units .The mean colony forming unit reduction was 96.14%which was found to be significant when analyzed using T tests followed by Wilcoxon Matched- Pairs signed –Rank test(P value<.001).

Table IV

Comparing the efficacy of 0.2% Chlorhexidine and 10% Povidone Iodine

Treating agent	**No.**	**Pretreatment**	**Post treatment**	**% reduction**	**Mean +_S.D.**	**P value**
0.2% chlorhexidine	**20**	**23075+_17912.47**	**2695+-1773.63**	**86.2%**	**86.2+_8.20**	**<.001****
10% Povidone Iodine	**20**	**7125+_3978.88**	**275+_286.31**	**96.14%**	**96.17+_ 4.21**	

Table IV shows the comparative evaluation of the antimicrobial efficacy of 0.2% Chlorhexidine and 10% Povidone Iodine which was represented by mean percentage colony forming unit reduction. Microbial efficacy of Chlorhexidine & Povidone Iodine were analyzed by T-tests followed by Mann-Whitney showed significant difference (P<.001).

Table V

Baseline contamination with different water reservoir sources

Different water sources	Mean values before passing through lines	No. of dental units	Cfu/ml after passing thru lines	Mean values +_ S.D. after passing thru the lines	P Value
Mineral water	500	5	4900 5600 4400 6300 5800	5400[a] +_ 751.66	<.001**
Sterile distilled water	0	5	3300 4500 3800 2200 5400	3840[a] +_1209.55	
Fresh tap water	1300	5	13800 12600 9800 11200 11000	11680[b] +_ 1546.61	

**** denotes significant at 1% level**

Different alphabets between source denotes significance at 5% level

Using Post Hoc tests

Mineral water Vs sterile distilled water -- . 195 Non significant

Mineral water Vs fresh tap water ---------- .000

Sterile distilled water Vs Fresh tap water -- .000

Table V shows the effect of different types of water when used as reservoir source on the baseline contamination of dental unit waterlines. No significant difference were found in mean baseline contamination using sterile distilled water and mineral water as

a reservoir source using Anova followed by Student Newman Keuls tests while mean baseline contamination using fresh tap water as reservoir source showed significant difference in baseline contamination when compared with other two groups. According to this table either mineral water or sterile distilled water can be preferred to fresh tap water as reservoir source in dental unit waterlines

Table VI

Baseline contamination from different water outlets

Outlets	Fresh tap water mean value	No.of dental units	After passing through the lines	Mean values after passing through the lines +_ S.D.	P value
Hand piece lines	**1500 cfu/ml**	**5**	**7800 9700 5400 11200 5600**	**7940+_ 2533.38**	**0.996 NS**
Air/water syringe lines	**1500 cfu/ml**	**5**	**6300 10200 6800 9600 7500**	**8080+_1728.29**	
Ultrasonic scaler lines	**1500cfu/ml**	**5**	**7300 8600 6200 12600 5400**	**8020+_2828.78**	

P value found to be non significant

Using Post Hoc tests

Hand piece line Vs Air/Water lines – 1.000 NS

Handpiece line Vs Scaler lines - 1.000 NS

Air/water syringe lines Vs Scaler lines- 1.000 NS

Table VI shows evaluation of baseline contamination of water collected from different water outlets of dental units. No significant difference were found in mean baseline contamination of water collected from different outlets of dental units such as Handpiece outlets , Air/ Water syringe outlets and scaler outlets with Anova followed by Post Hoc tests.

Table VII

Duration of efficacy of 0.2% Chlorhexidine

Treating agent	Baseline Values in 5 dental units	After treatment	3rd day	5th day	7th day	P Value
0.2% chlorhexidine	11600 9600 14000 8300 6500	1200 1100 1000 800 400	2800 4500 4400 2300 2600	6400 7600 9800 4900 5800	9800 8700 11200 7600 8400	
Mean +_ S.D.	9880.00[d] +_2838.49	900.00[a] +_316.23	3320.00[b] +_1047.38	6900[c]+_ 1894.73	9140[cd] +_ 1395.71	<.001**

Using paired samples tests

Baseline Vs after treatment –-- .002

Baseline Vs 3rd day --------- .003

Baseline Vs 5th day ---------- .015

Baseline Vs 7th day ---------- .383 Non significant

Table VII shows the duration of the efficacy of 0.2% Chlorhexidine in reducing the microbial contamination of dental unit waterlines. The mean colony forming units on 3rd (3320) ,5th (6900)and 7th day(9140) after treatment was statistically analyzed using Kruskal-Wallis 1-way Anova test showed significant difference. There is a gradual increase in colony forming units reaching the baseline values by 7th day.

Table VIII

Duration of efficacy of 10% Povidone Iodine

Treating agent	Baseline	After treatment	3rd day	5th day	7th day	P Value
10% PI	14000 14000 3200 4400 2800	300 400 100 200 100	300 500 200 200 200	500 900 1100 600 300	5400 7500 3300 2600 2800	<.001**
Mean +_ S.D.	7680.00[b] +_5799.31	220.00[a] +– 130.38	280.00[a] +–130.38	680.00[a] +_319.37	4320.00[ab] +–2096.90	

Using paired samples tests

Baseline Vs after treatment -- .042

Baseline Vs 3rd day ---------- .044

Baseline Vs 5th day ---------- .054

Baseline Vs 7th day ---------- .131 NS

Table VIII shows the duration of the efficacy of 10% Povidone iodine in reducing the microbial contamination of dental unit waterlines. The mean colony forming units on

3rd day(280) ,5th day (680) after treatment was statistically analyzed using Kruskal-Wallis 1-way Anova test showed no significant difference. There is a gradual increase in colony forming units reaching the baseline values by 7th day (4320cfu/ml)

Table IX – comparing the duration of efficacy of 0.2% Chlohexidine & 10% Povidone Iodine

Treating agent	Baseline	After treatment	3rd day	5th day	7th day
0.2% chlorhexidine	9880+_2838.49	900+_316.23 (90.89%)	3320.00 +_1047.38 (66.39%)	6900+_ 1894.73 (30.16%)	9140 +_ 1395.71 (7.48%)
10%Povidone Iodine	7680+_5799.31	220+_+-130.38 (97.13%)	280.00 +-130.38 (96.35%)	680.00 +_319.37 (91.145%)	4320.00 +-2096.90 (43.75%)
P value		.661	.0152	.000	.000

Table IX compares the duration of the efficacy of 0.2% Chlorhexidine versus10% Povidone iodine. Immediate post treatment values with both the agents, showed no significant difference. Difference were observed between 3rd mean cfu/ml 3320, 280), 5th day (6900,680) and 7th day(9140, 4320) sample mean contamination, 10% Povidone iodine was found to be more efficient (97.13%) and active for a period of 3 days (mean cfu 280) and gradually loosing its efficacy by 7th day.

DISCUSSION

The provision of dental unit water that is safe for use with all categories of patients is now an issue world wide. Microbial quality of dental unit water is important to both patients and staff as they are exposed to water and aerosol generated by dental unit.[28] Dental procedures might expose patients and dental professionals to opportunistic and pathogenic organisms originating from the various components of the dental unit, which might be potential for human impact.[6] Dental practitioners should be aware of this issue as it can cause various infections and also result in cross infections. Martin in 1987[39] has reported two postoperative infections caused by Pseudomonas aeruginosa that were believed to have originated from dental unit water. People considered to be at risk are elderly people and immuno compromised persons like HIV/AIDS patients, patients with chronic auto immune diseases/ organ transplant recipients, patients on prolonged radiotherapy ,patients with multiple blood transfusions, patients exposed to immunosuppressive agents .

The phenomenon of microbial colonization of dental water delivery systems was first reported by Blake in 1963[5].He was the first to test the effectiveness of chemical germicides as a possible solution to the problem. The subsequent investigations by a number of researchers led the Center for Disease Control and Prevention to first address the topic of water quality in its 1993 infection control guidelines. A recommendation has been issued by American Dental Association[1], that is by the year 2000, water delivered to patients during non surgical dental procedures consistently contains no

more than 200 colony forming units/ml of aerobic, mesophilic, heterotrophic bacteria at any point of time in the unfiltered output of dental units.

Dental unit waterlines are considered to be the integral part of dental units as they supply water to air turbines and ultrasonic scalers as a coolant. Dental unit water lines are very small in diameter; present a very high surface-to volume ratio with relatively low flow rates, intermittent patterns of use and overnight stagnation that are ideal for colonization with aquatic bacteria, leading to biofilm formation.[8, 9]

Biofilms are microbial communities that adhere to solid surfaces wherever there is sufficient moisture (including plant and animal tissues) consisting primarily of bacteria. Biofilms also provide an environment conducive to the proliferation of a wide variety of other microscopic life, including fungi, algae, protozoa and nematodes that are enveloped in a polysaccharide slime layer known as a glycocalyx. The glycocalyx protects the organisms within from desiccation, chemical insult and predation, as well as from attacks by plant and animal immune systems[54].

The Dental unit waterlines biofilm is a mixture of living bacteria, extracellular carbohydrates and biological debris, once established , bacterial cells are continuously recruited to and released from the biofilm into the walls flowing through or standing in the tubing lumen. Two problems can arise from the presence of biofilms in a distributing aqueous system. First, the biofilm can clog pipes and tubings or interfere with the proper function of mechanical devices. Second, bacterial populations living in this protected mode of growth produce planktonic cells that contaminate fluids and alter their properties or, in the case of pathogens, can result in various infections.[25]

If untreated, the microbial populations in dental unit waterlines often exceed 10^4 to 10^5 colony forming units /ml of water[35] .Despite the lack of epidemiological data demonstrating a positive correlation between contaminated Dental unit waterlines and significant patient health problems, it has been suggested that the dental profession should take proactive steps to limit microbiological contamination of water used in all forms of dental treatment.[10]

Currently, there are several methods of reducing the numbers of colony forming units in the Dental unit waterlines, including flushing lines with water, intermittent or continuous use of bactericidal chemicals, radiation, self-contained independent water reservoirs, and filtration[54] .

The recommendations of the U.S. Centers for Disease Control and Prevention (CDC), the American Dental Association (ADA) and the British Dental Association (BDA) are that waterlines should be flushed through for "several minutes" at the beginning of each clinical day to expel the overnight build up of microbial load in stagnant areas and for 20–30 seconds between patients to remove material that may have been retracted during treatment.[54]

According to Cobb[10] time-dependent flushing for as few as two to four minutes would produce a statistically significant reduction in planktonic bacterias as compared to baseline and the level of colony forming units /ml after 4minutes of flushing still exceeded the current American Dental Association recommendations for acceptable level of microorganisms. Although these extended flushing times resulted in large drops in colony forming units, flushing for excessive time periods is impractical in large, multichair treatment clinics.

Another method of reducing the waterline contamination is Filtration. To be effective, filters must be located on each water-bearing line as close as possible to the handpiece or the air–water syringe. The filters do not affect the flow rate of water to any significant degree and have no impact on biofilm formation[8] .According to Murdoch-Kinch 1997 [7] high levels of recontamination of Dental unit waterlines occur within 24 hours as a result of trapping and growth of bacteria on filters. Therefore, disposable filters are recommended and must be changed daily [8] but their clinical effectiveness has not yet been fully established [16].

According to Franco[16], Anti-retraction valves (also known as check valves) are now used to prevent re-aspiration of contaminated fluid and hence reduce the risk of transfer of potential infective material. However, it seems that the majority of the antiretraction devices do not prevent retraction when the turbine stops running, particularly after it has been used for some time, and this leads to a contamination of the waterlines. Also, as with other components of the water supply line, the valves are subject to clogging owing to biofilm deposition and fatigue. In order to ensure adequate mechanical functioning they require regular maintenance and programmed replacement (Pankhurst 1998)[8].

Treatment with various biocides are recommended by many authors in reducing the microbial contamination of dental unit waterlines [25 , 54, 59]. The largest number of studies of waterline treatment that had been published since 1963, have investigated various chemical agents intended to inactivate microorganisms, induce detachment of biofilms

or both. The second largest studies examined the flushing of waterlines and the use of filters received the least attention[54]

Blake in his 1963[5] article had given the recommendations for antiseptic solutions. According to him it should be a very dilute solution and should be effective against a wide range of microorganisms. It should have pleasant flavor and in effective concentration should not be detrimental to tissue or react with metal parts of the apparatus. According to Mills 2000[54] an ideal agent for chemical treatment should control biofilm and should be bactericidal but not toxic or irritating to humans. It should detach biofilm and discourage subsequent reformation, while protecting the dental unit's internal components from corrosion or degradation. If delivered continuously in treatment water, it should have no effect on enamel or dentin bonding agents. It should be inexpensive and easy to use. Chemical treatment can be of two types: continuous and intermittent.

Continuous treatment uses either lower concentrations of potentially biocidal agents or less toxic (biostatic) substances in the water used for patient treatment. It also might employ initial shock treatment to inactivate or eliminate biofilms. Although it offers less potential for recolonization of waterlines, still may damage the equipment. Since the agent is always present and may be aerosolized, the effects of chronic exposure on the health care worker must be considered. Enamel and dentin bond strength of dental adhesive materials also may be affected. According to Nuala B. Porteous 2003[42] study treatment of Dental Unit waterlines with a continuous use waterline cleaner might alter the natural water flora and promote the growth of a fungus that is already present in small amount in the water source.

Intermittent treatment regimens use potentially biocidal concentrations of germicide that may remove biofilm .This approach is called by the expert as "shock treatment." Usually the agent will be delivered for a specified contact time and frequency using an independent water reservoir that isolates the unit from the municipal water supply. This also permits the use of water of known microbiological quality for subsequent therapeutic procedures. A major advantage of intermittent chemical use is that the active agent is purged from the system before patient treatment and disadvantages include the potential for surviving biofilm organisms to rebound between treatments, Staff exposure to chemicals, adverse impact on metal, rubber and synthetic dental unit components. The disadvantages can be minimized by using effective treating agent which has maximum potency with minimal side effects.

Hence this study was undertaken in the department of Pedodontics & Preventive dentistry, Ragas Dental College, to enumerate and identify the microorganisms present in water samples collected from dental unit waterlines of different specialty clinics and to find out the efficacy of two commonly available treating agents in disinfecting dental unit waterlines.

Dental units in the study were selected which had been in daily use for approximately one year and had never been treated for the removal of biofilm or reduction of planktonic bacteria.[36]

As a part of laboratory procedure, to evaluate the number of heterotrophic microorganisms in each water sample in the study used spread plate technique with a low nutrient R2A agar, suggested by Laura Noce[34]. The nutrient medium conforms with recommendations of the standard methods ((US-EPA) for the examination of water

and is suitable for the recovery of stressed and chlorine tolerated bacteria in combination with a low incubation temperature and an extended incubation time. The low concentration of yeast extract, casein hydrolisate, peptone and glucose allows a wide spectrum of bacteria to grow without the fast growing bacteria suppressing slow growing species.

Enumeration was done with the help of magnifying glass as in the study done by Barbeau[25], by counting the total colony forming units irrespective of the type and genera and their concentration reported as colony forming units in 1ml of water – colony forming units /ml which was calculated by number of colonies x dilution factor[37] . The colonies grown were first assessed macroscopically considering characteristics such as size, shape, structure, colony color followed by microscopic methods for identification of the microorganisms by Gram method[32] . According to Mills[54] most organisms recovered from dental water systems are gram negative noncoliform bacteria. Further tests like sugar reactions[5], oxidase tests [2,25] were carried out for confirmation of the microorganisms cultured.

Disinfectants were selected in the study according to their ability to kill microbial cells & remove biofilm from the inner surfaces of Dental unit waterlines tubing according to the study outcome of Walker 2003 [59].

Sodium hypochlorite (5.25% diluted 1:10) was the most commonly used disinfectant for dental unit waterlines. But research has proved that they give away by-products like trihalomethanes, which have hazardous effects on the human tissues and can corrode metal component[45]

Gluteraldehyde is a highly effective disinfectant with bactericidal action against most vegetative bacteria, mycobacteria and viruses but its sensitization of the human lung and skin limited its use .[8]

Commercial products available are Alpron BRS solution (Sodium hypochlorite , citric acid), Alpron Mint (Sodium –p-toluol-sulfonechloramide , EDTA), Bioblue (Ethanol, Chlorhexidine) , Dentosept P (Hydrogen peroxide , silver ions), Oxygenal 6 (Hydrogen peroxide , silver ions), Sanosil Super 25 (Hydrogen peroxide , silver ions), Sterilex Ultra (Alkaline peroxide), Ster4 spray (Sodium perborate , EDTA). Alpron caused foaming, staining and brown discoloration of water. Ster4spray and Sterilex Ultra caused blocking in some Dental Unit Water systems [51]. According to Schel et al 2006 dental practitioners must consult with the manufacturer of their Dental Unit Water Systems prior to introducing any of these chemical agents.

Most of the cleaners and disinfectants do not effectively remove the biofilm because the biofilm carry a net negative charge which results in repulsion or non interaction of materials. Chlorhexidine was selected as one of the treating agents, which is a positively charged organic antiseptic agent belongs to the bis –biguanides group. Second treating agent selected was10% Povidone Iodine which is a highly efficient microbicide to a wide variety of bacterial, fungal and viral infections. Even though it has disadvantages of generating iodophor laden aerosols [52] and elevation of dissolved mercury levels in dental unit waste water [38],because of its known antibacterial efficacy and relative lack of toxic or irritating properties , Povidone Iodine was selected. Treatment procedure was carried out intermittently in the lines as conducted in Naula study[43].

The water samples of the study showed bacterial colony count varied from 2800 colony forming units /ml to a maximum of 68000 colony forming units /ml with a mean colony forming units of 18380.28/ml, which was found to be higher than the American Dental Association recommendation level of 200 colony forming units /ml and none of the dental units under study delivered water that could meet the accepted standard for potable water.

Mills 1986[52] study could identify $7x10^{3}$ to $5x10^{5}$colony forming units /ml of microorganisms with a mean of $4.5x\ 10^{4}$ colony forming units /ml. Jolanta Szymanska 2007[32] study , showed that the concentration of bacteria isolated was found to be minimum of 22,300colony forming units /ml to a maximum of 5,85,000 from the 25 dental unit reservoirs.

The study by Souza –Gugelmin et al[37] showed the bacterial concentrations found in dental unit water ranged from 0 to $1.52x\ 10^{6}$ colony forming units /ml. These variations were explained by Barbeau et al 1996[25] as the heterogenous distribution of bacterial cells within a given water sample. Bacterial cells in the water obtained from Dental unit waterlines are thought to be released from the biofilm formed inside the tubing. During sampling, small pieces of biofilm or microcolonies may be released. This is likely to result in a bias toward higher or lower counts or toward the predominance of a given bacterial species in the sample.

The cultures from water samples showed the presence of following microorganisms in the descending frequency- Pseudomonas 70 (98.59%), Staphylococci 65 (91.55%) , Klebsiella 26(36.62%),Candida 25 (35.21%), Bacillus 24(33.8%), Serratia 22 (30.99%), Proteus 14 (19.72%),Methylobacterium Mesophilicum 5 (7.04%) ,E. Coli 4 (5.63%)

Barbeau[25] in 1996 in his study on multiparametric analysis of waterline contamination in dental units, Sphingomonas paucimobilis and Acinetobacter calcaceticus were the predominant cultivable species found in the microflora of Dental unit waterlines. The opportunistic pathogen Pseudomonas aeruginosa was isolated from 24% of examined units. Dental units contaminated by Pseudomonas aeruginosa showed significantly higher total bacterial counts than others. Less predominant species obtained in the isolates from dental units were identified as: Pseudomonas maltophilia, Pseudomonas putida, Pseudomonas fluorescens, Pseudomonas vesicularis, Pseudomonas acidovorans, Actinomyces species and Bacillus species . Some yeasts and amoebae were observed by direct microscopic observation.

Meiller[56] in his study isolated Burkholderia pickettii, Burkholderia cepacia, Psychrobacter phenylpyruvica, Moraxella osloensis, Sphingomonas paucimobilis, Myroides odoratum, Brevundimonas vesicularis, Achromobacter species, Stenotrophomonas maltophilia, Staphyloccoccus species, Bacillus species, Pseudomonas stutzeri, and Alcaligenes faecalis (odorans). Opportunistic and true human pathogens such as Pseudomonas aeruginosa, Legionella pneumophila, Mycobacterium species and Staphylococcus species also been isolated.

Only Pseudomonas aeruginosa derived from Dental unit waterline has definitively been reported to have given rise to infections in two immunocompromised patients [39]. Al-Hiyasat et al[2] sudy in 2007 percentage of Pseudomonas was found to be 86.7%. Fresh tap water in the study also showed the presence of Pseudomonas .The source of the contamination could not be due solely to the water source as Pseudomonas is present in the oral cavity and can be aspirated back from the mouth into the dental unit

waterline through a defective check valve.[25] Methylobacterium Mesophilicum was detected in Barbeau et al 1996 study in 19% of samples. Blake in his 1963[5] study isolated Klebsiella aerogenes, Bacillus Subtilis, Pseudomonas Pyocyanea.. Proteus was isolated in Franco[16] study .Confluent growth of coliform organism in 63.1% of samples were found in Szymanska[28] study .The percentage of isolated staphylococci in the study was found to be higher than that of a recent study carried out by Szymanska 2007[32] in which he pointed out that staphylococcus genera which form the physiological flora of the oral cavity, were present in Dental unit waterlines , might be as a result of sucking back fluids from patient's oral cavities and subsequent multiplication in the unit reservoir. Candida species was detected in walker et al 2000[23] study.

Reduction in microbial contamination after using the treating agent 0.2%Chlorhexidine was found to be ranged from 68% to 92.54% and mean reduction was 86.2% in the study. This percentage reduction was observed to be statistically significant, when analyzed using Paired T tests followed by Wilcoxon Signed Rank tests (<.001**)

Chlorhexidine has its primary effect on bacterial cell membrane. At low concentration, it causes cellular constituents to leak from the cell and at high concentration it results in a precipitation of cell membrane and cytoplasmic constituents. It has a broad spectrum antibacterial effect by virtue of its high intra-oral substantivity .In the oral environment it has both bacteriostatic and bactericidal properties and mechanisms of action are multifactorial. Electrostatic interactions occur between Chlorhexidine and the oral tissues which are both reversible and pH dependent, thus allowing Chlorhexidine to be released over a period of time, preventing

multiplication and adherence of organisms[43] . It is not significantly neutralized by soaps, body fluids or other organic compounds. The solution is near-neutral (pH range 5–7). Chlorhexidine gluconate is a salt of Chlorhexidine and gluconic acid. Its molecular formula is C22H30Cl2N10•2C6H12O7.

Walker et al [59], in his study, compared the efficacy of a variety of products based on the different classes of active compound. According to him, 0.2% Chlorhexidine reduced the viable count by100% and biofilm coverage was 31.77%. Gram negative bacteria with resistance to certain antibiotics also showed increased Chlorhexidine resistance.[43] Resistance to some disinfectant can be provided to fixed bacteria by deactivation of disinfectants upon contact with underlying surface or deposits mixed with biofilm. Nuala B.Porteous (2004)[43] performed a study to test the efficacy of an intermittent use, dental unit waterline cleaner containing 0.12% chlorhexidine to reduce the bacterial levels in three functional units with independent water reservoir systems and found significant reduction of bacterial counts to levels that were consistently below American dental association's goal of 200colony forming units /ml for 8 weeks.

The treatment with 10% Povidone iodine showed the reduction in microbial contamination ranging from 83.60% to 100%. The mean value was 96.14% which was found to be significant when analyzed using T tests followed by Wilcoxon Matched- Pairs signed –Rank test(P value<.001). It is highly efficient microbicide against a wide variety of bacterias, fungi and viruses. It is formed by binding free iodine to poly vinyl – pyrrollidone, a solubilising agent. This is done to decrease the toxicity of iodine. As iodine is liberated from the Poly vinyl –pyrrollidone molecule, it exerts its antimicrobial effect. Once released, iodine is toxic to microorganisms because it

combines irreversibly with tyrosine residues of proteins, interferes with the formation of hydrogen bonding by some amino acids and nucleic acids, oxidizes sulfydryl groups and reacts with the sites of unsaturation in lipids[33]. Povidone iodine is water soluble, does not irritate healthy or diseased oral mucosa and exhibits no adverse side effects such as discoloration of teeth and tongue and change in taste sensation[55]. Mills et al.[52] suggested that 10% Povidone iodine be used to reduce microbial contamination. Walker et al study 10% Povidone iodine reduced the viable count by 100% and biofilm coverage was 97.3%. After treatment with Povidone iodine, in the study Pseudomonas was found to be resistant organism in 16 samples out of 20.

When compared the antimicrobial efficacy of 0.2% Chlorhexidine with 10% Povidone Iodine showed less mean percentage colony forming unit reduction (86.2% vs 96.14%) which were analyzed by T-tests followed by Mann-Whitney showed significant difference (P<.001).

When different types of water was used as reservoir source on the baseline contamination of dental unit waterlines, no significant difference were found in mean baseline contamination using sterile distilled water and mineral water as a reservoir source using Anova (P .195) followed by Student Newman Keuls tests while mean baseline contamination using fresh tap water as reservoir source showed significant difference in baseline contamination when compared with other two groups (P<.001). According to the study results either mineral water or sterile distilled water can be preferred to fresh tap water as reservoir source in dental unit waterlines **.**A study by Kettering et al[21] compared using combinations of tap water & sterile distilled water with or without two chemical disinfectants over a six week period in 75 new dental units. He

concluded that water source selection plays an important role in achieving and maintaining consistent disinfection of dental unit waterlines and tap water should not be used as a water source for Dental unit waterlines. But Nuala Porteous in 2004[43] in their study suggested that in institutions with large numbers of functioning operatories, this may not be practical or cost effective measure.

The evaluation of baseline contamination of water collected from different water outlets of dental units showed no significant difference in mean baseline contamination of water collected from different outlets of dental units such as Handpiece outlets, Air/ Water syringe outlets and scaler outlets with Anova followed by Post Hoc tests (P 1.000 NS).In Barbeau et al study difference in microbial contamination were found between water from the turbine and air/water syringe. Air water syringe connected waterlines yield lower bacterial counts than high speed drill even though material & diameter are the same. [25] According to him it can be due to different flow rates and air water syringe used more frequently in a day to day basis. In Al – Hiyasat et al [2]study in 2007, water collected from the air/water syringe outlets were found to be more contaminated than handpiece lines. Szymanska J in 2005[29] conducted a study using electron microscope to detect the presence of biofilms on the inner surfaces of the tubing in dental unit waterlines. Samples for examination were taken from the tubes providing water to high-speed and slow-speed handpieces, and to an air-water syringe before application of a disinfection procedure and no significant differences were found in the bacterial biofilm between high-speed, slow-speed and air-water lines.

Mayo 1990[40] study examined the bacteriology of dental air-water syringes, and found that the water delivered by these syringes can be persistently contaminated with

bacteria. Flushing of the water line reduced but did not eliminate this contamination. Even after six minutes' flushing, some water samples still contained more than 10^4 viable bacterial cells per milliliter, although coliform counts were less than two per 100 milliliters .Sterilization of the tip or the entire syringe did not eliminate this contamination. Scanning electron microscopy revealed bacterial biofilms on the inner wall of the plastic tubing supplying water to the air-water syringe, but not on the air line or on new, unused tubing.

When the duration of the efficacy of 0.2% Chlorhexidine in reducing the microbial contamination of dental unit waterlines was checked, the mean colony forming units on 3rd (3320) ,5th (6900)and 7th day(9140) after treatment was statistically analyzed using Kruskal-Wallis 1-way Anova test and it showed a significant difference. There is a gradual increase in colony forming units reaching the baseline values by 7th day. Even though the microbial contamination is found to decrease effectively it did not match the American Dental Association recommendation level. According to the study results 0.2% Chlorhexidine should be used on daily basis .When the duration of the efficacy of 10% Povidone iodine in reducing the microbial contamination of dental unit waterlines were evaluated the mean colony forming units on 3rd day(280), 5th day (680) after treatment were found which was statistically analyzed using Kruskal-Wallis 1-way Anova test showed no significant difference. There is a gradual increase in colony forming units reaching the baseline values by 7th day (4320colony forming units /ml).

Shannon E. Mills, Patricia W. Lauderdale, Robert B. Mayhew (1986)[52] evaluated the reduction of microbial contamination in dental units with Povidone iodine 10%. Undiluted Povidone iodine 10%, loaded in five experimental units for 12 hours

prevented the recovery of microorganisms for 3 to 14 days when used in combination with sterile water reservoirs. Use of sterile water reservoirs alone did not effectively reduce the levels of microbial contamination in five control dental units. In the present study mean colony forming units on 3rd day is near the value of American Dental Association recommendation after that it showed gradual increase in microbial contamination. According to the study results 10% Povidone Iodine should be used every 3rd day as a treating agent in dental unit waterline.

An analysis of the results of the study shows that the use of a water source with the counts within the environmental protection agency drinking water standard of 500colony forming units /ml is mandatory to begin with. To continue maintaining the sterility of the Dental unit waterlines and to complete the infection control measures adopted in the dental clinics ,suitable disinfectants like 0.2% Chlorhexidine on daily basis or 10% Povidone iodine on every 3rd day basis intermittently are recommended .

The present study included the evaluation of efficacy and duration of the action of irrigants for one week period and also different reservoir water sources. However due to practical difficulties effect on biofilm coverage, adverse effect on the waterline tubing , byproduct formation when treating agents are used intermittently ,the effect of Chlorhexidine and Povidone Iodine on the enamel and dentin bond strength of dental adhesive materials and the development of resistance to these treating agents when used for prolonged period of time could not be assessed. Future clinical research in this field can be undertaken to overcome these limitations and to arrive at more specific recommendations for maintenance of the sterile environment in the dental clinic.

SUMMARY

This study was carried out in the department of pedodontics &preventive dentistry to enumerate and identify the microorganisms present in water samples collected from dental unit waterlines of different dental speciality clinics and to find out the efficacy of two treating agents in disinfecting dental unit waterlines. Sample included 70 dental unit waterlines from different speciality dental clinics which were checked for microbial contamination. From these dental units 40 units were randomly selected and divided into two groups of 20 each. Group A, treatment was done in20 dental units with 0.2% Chlorhexidine gluconate solution and Group B ,treatment was done in 20 dental units with 10% Povidone iodine solution and the reduction in the microbial levels were assessed. Five dental units were randomly selected and checked the microbial contamination using mineral water, sterile distilled water, fresh tap water as a water source in the dental unit reservoir bottles. Five dental units were randomly selected to collect water samples from three different water outlets such as handpiece lines, air/water syringe and scaler lines and microbial contamination were checked. Also from the test group, five from each group were checked for the duration of efficacy of treating agent for one week by analyzing the water samples collected on 3**,** 5 and 7 day intervals. From the results we can infer that most of the identified microorganisms comprise of Gram negative and pseudomonas predominating up to 98.59% of the total isolates. Usage of disinfectants 0.2% Chlorhexidine and 10% Povidone Iodine were found to be very

effective in reducing the microbial contamination and 10% Povidone iodine was found to be more efficient (97.13%) and active for a period of 3 days and gradually loosing its efficacy by 7th day. No significant difference were found in microbial contamination of water samples collected from different water outlets such as handpiece outlets, air water syringe outlets, scaler lines. According to the study results either mineral water or sterile distilled water can be preferred to fresh tap water as reservoir source in dental unit waterlines. Therefore to maintain the sterility of dental unit waterlines it is essential to have a good water source and an effective disinfectant.

CONCLUSION

1) All the test dental units showed microbial contamination which was polymicrobial in nature.
2) Most of the identified microorganisms comprise of Gram negative bacteria and Pseudomonas (98.59%) was found to be the most prevalent organism.
3) 0.2% Chlorhexidine showed 86.2% reduction in the microbial contamination
4) 10% Povidone Iodine showed 96.14% reduction in microbial contamination
5) 10% Povidone Iodine was found to be more effective compared to 0.2% Chlorhexidine ($P<0.001$)
6) Mineral water or sterile distilled water preferred to fresh tap water as a reservoir source
7) No significant difference was found in microbial contamination of water samples collected from different water outlets such as handpiece outlets , air water syringe outlets , scaler lines (P value 0.996)
8) 0.2% Chlorhexidine can be used as an effective treating agent on daily basis
9) 10% Povidone Iodine can be used as an effective treating agent on every 3rd day basis for microbial control.

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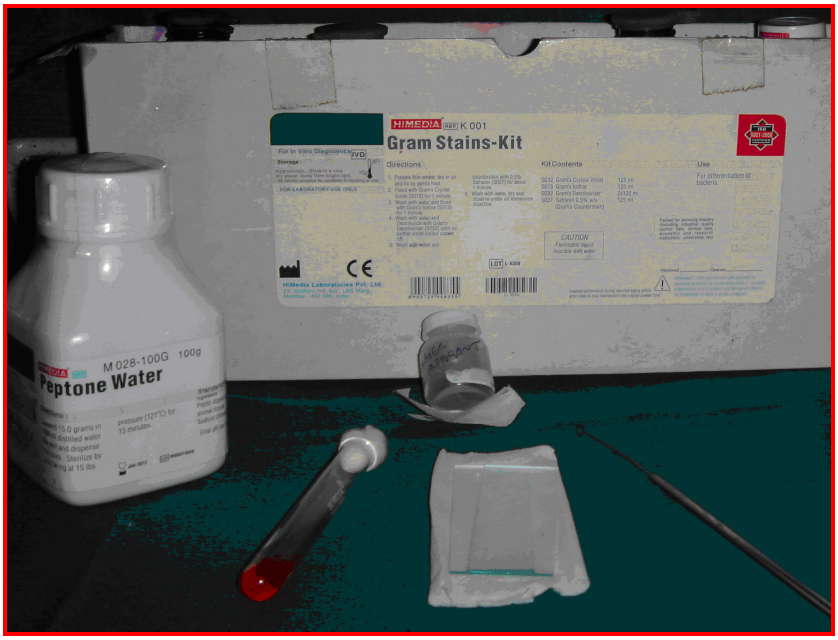
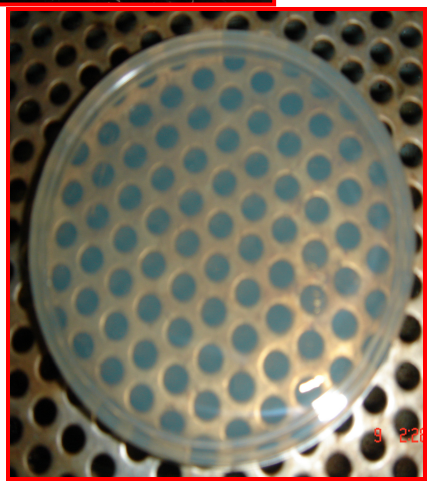
MATERIALS


DISTILLED WA
SURGITA
SPIRI
500 ml
Stock A
Stock B
NaH2PO4
Na2HPO4


HIMEDIA K 001
Gram Stains-Kit
Directions
Kit Contents
Use
CAUTION
M 028-100G 100g
Peptone Water

PREPARATION OF R2A AGAR MEDIA



METHODOLOGY

COLLECTION OF WATER SAMPLE USING STERILE TECHNIQUES

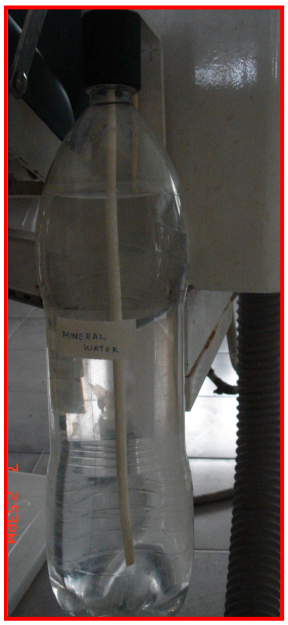
RESERVOIR BOTTLE FILLED WITH FRESH TAP WATER & REATTACHED TO THE UNIT

FLUSHING THE LINES

COLLECTION OF WATER SAMPLE IN STERILE BOTTLE

COLLECTION OF WATER SAMPLES WITH DIFFERENT WATER RESERVOIR SOURCES

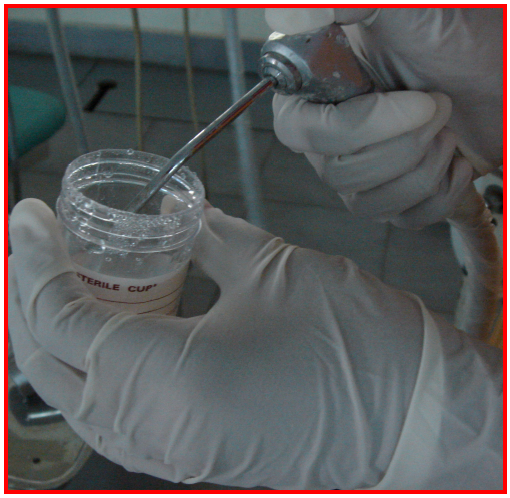
STERILE DISTILLED WATER



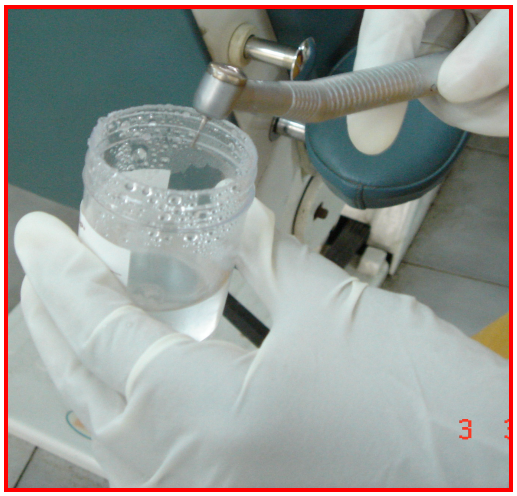
MINERAL WATER

FRESH TAP WATER

COLLECTION OF WATER SAMPLES FROM DIFFERENT WATEROUTLETS



Air water syringe outlets

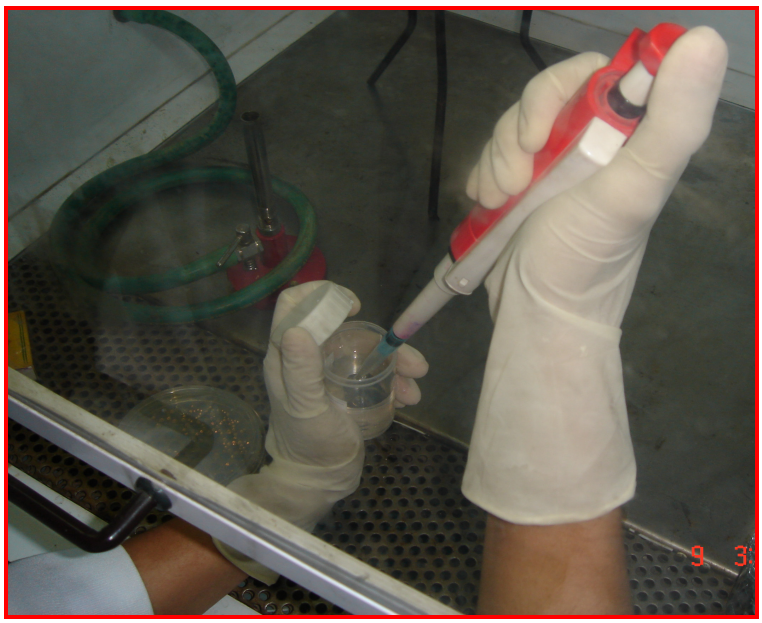


Handpiece outlets

Scaler outlets

WATER SAMPLES

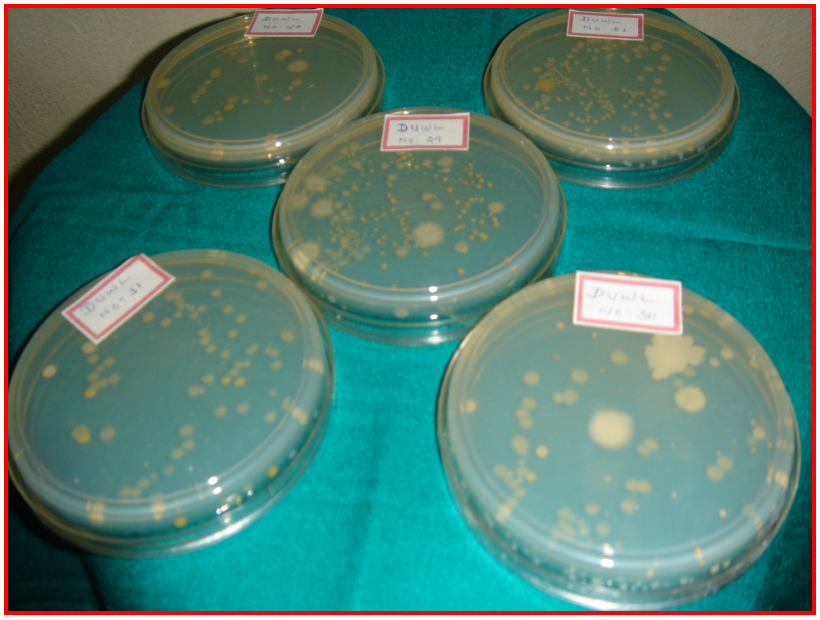
PREPARING THE DILUTION

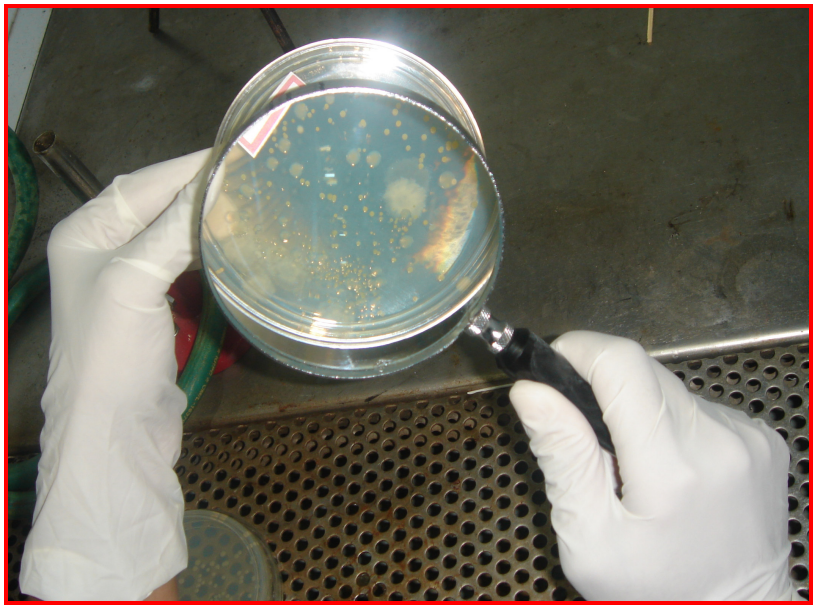


SAMPLE CULTURING (SPREAD PLATE METHOD)

INCUBATION AT 37^0 C FOR 5 DAYS

R2A AGAR PLATES SHOWING MICROBIAL GROWTH





IDENTIFICATION OF MICROORGANISMS

CONFIRMATORY BIOCHEMICAL TESTS

OXIDASE TESTS

TSI TEST

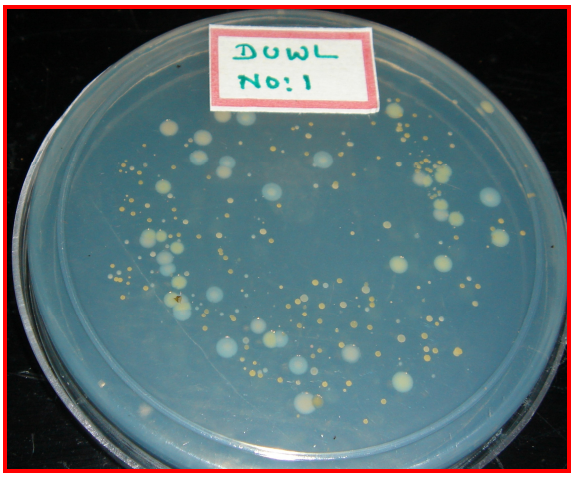
TREATING THE LINES WITH 0.2% CHLORHEXIDINE &
10% POVIDONE IODINE

0.2% Chlorhexidine

10% Povidone Iodine

RESULTS

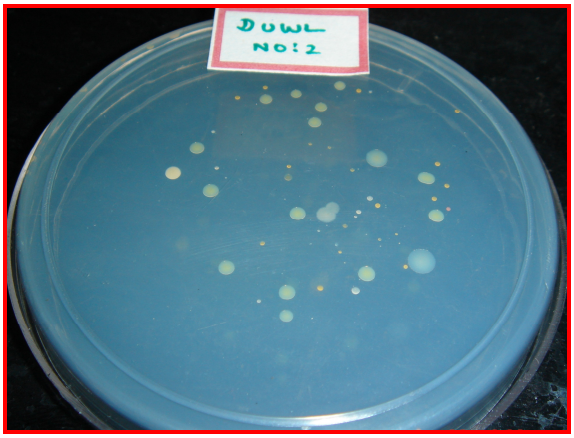
CULTURE PLATES SHOWING REDUCTION IN MICROBIAL CONTAMINATION WITH 0.2% CHLORHEXIDINE



PRETREATMENT

POST TREATMENT

CULTURE PLATES SHOWING REDUCTION IN MICROBIAL CONTAMINATION AFTER TREATMENT WITH 10% POVIDONE IODINE

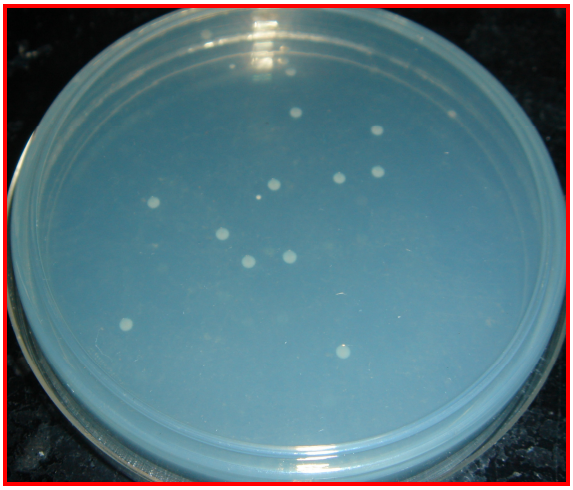


PRETREATMENT

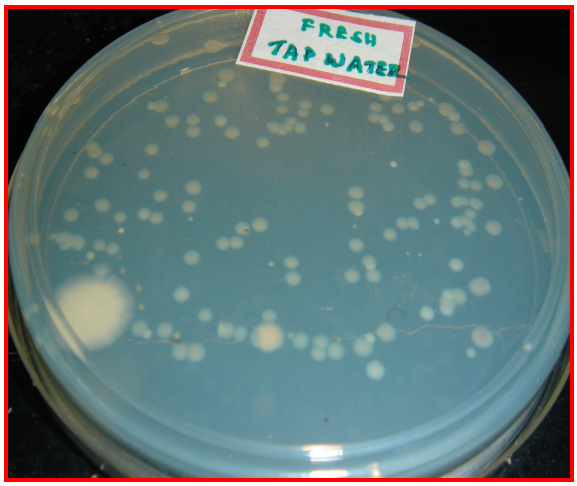
POST TREATMENT

CULTURE PLATES SHOWING MICROBIAL CONTAMINATION WITH DIFFERENT WATER SOURCES

FRESH TAP WATER

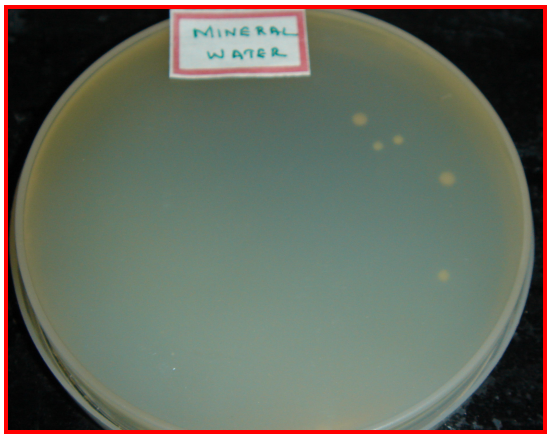


BEFORE PASSING THROUGH THE LINES

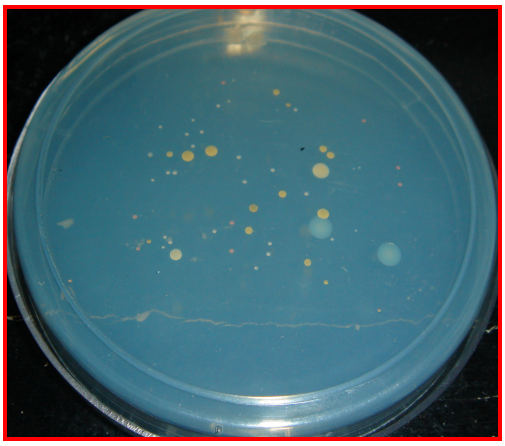


AFTER PASSING THROGH THE LINES

MINERAL WATER

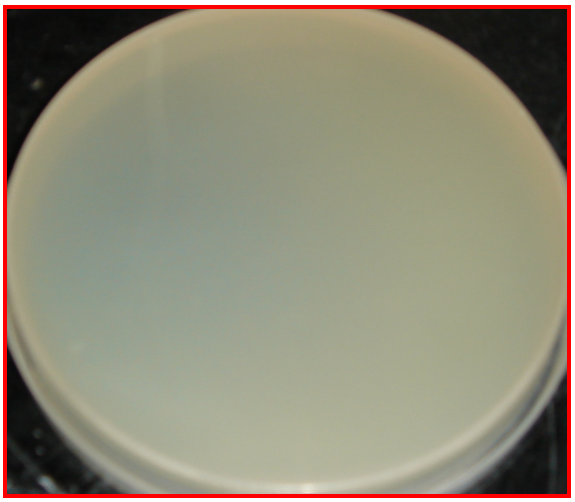


BEFORE PASSING THROUGH THE LINES

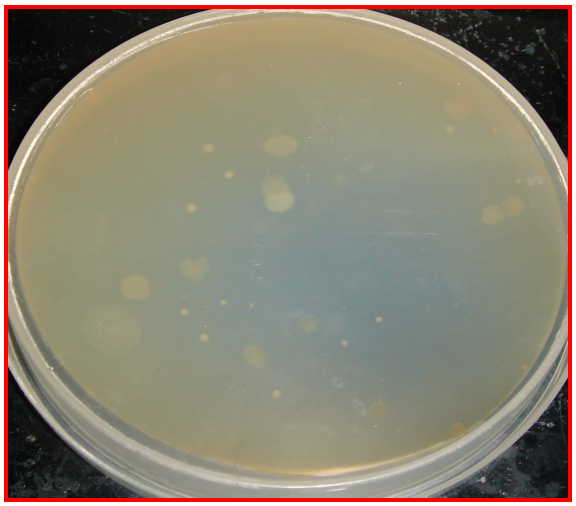


AFTER PASSING THROGH THE LINES

STERILE DISTILLED WATER



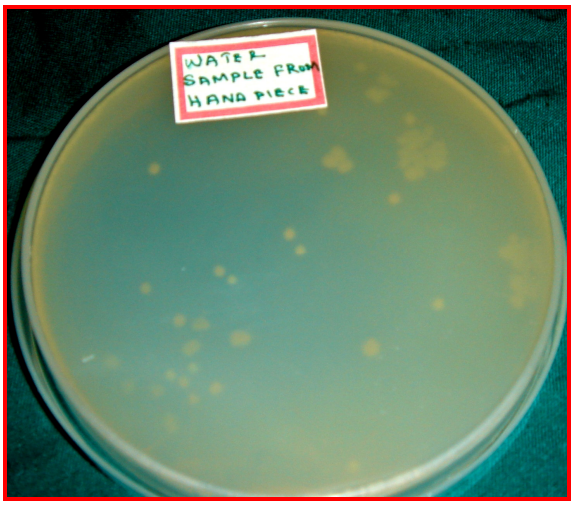
BEFORE PASSING THROUGH THE LINES



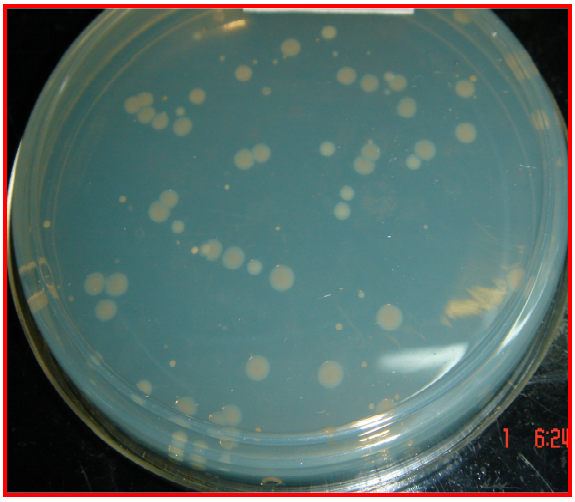
AFTER PASSING THROGH THE LINES

CULTURE PLATES SHOWING MICROBIAL CONTAMINATION WITH DIFFERENT WATER OUTLETS

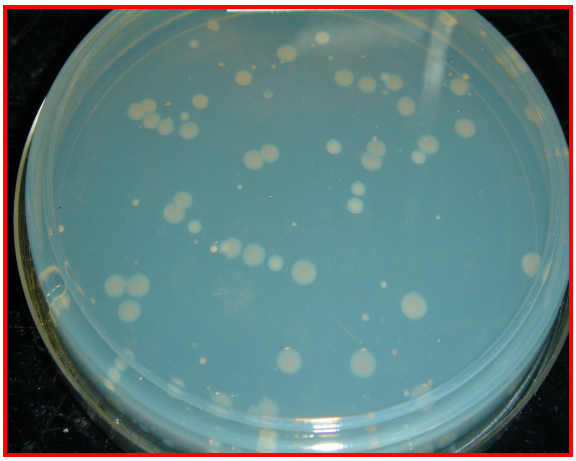
HANDPIECE OUTLETS



SCALER OUTLETS

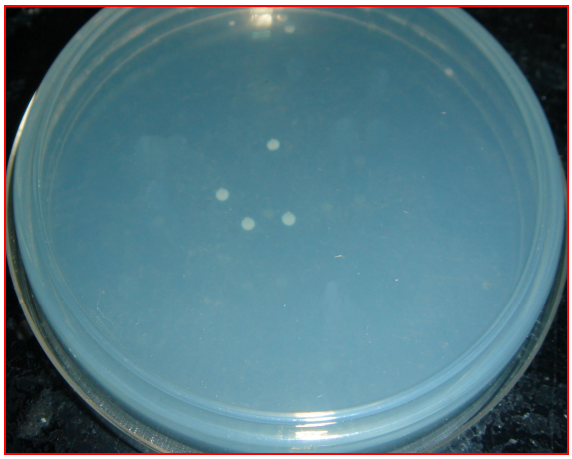


AIR WATER SYRINGE OUTLETS

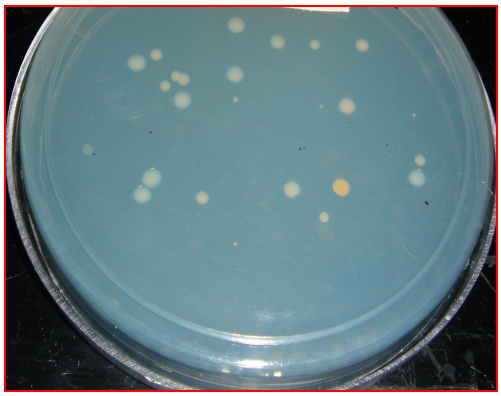


CULTURE PLATES SHOWING REDUCTION IN THE MICROBIAL CONTAMINATION WITH 0.2% CHLORHEXIDINE

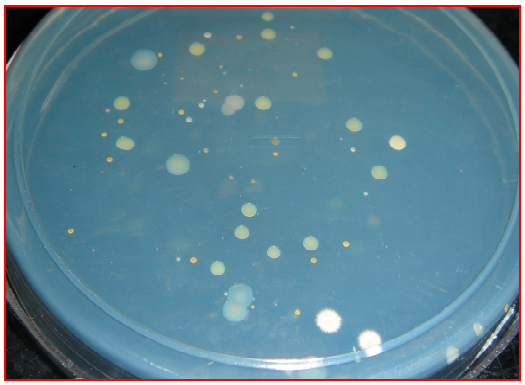
BASELINE



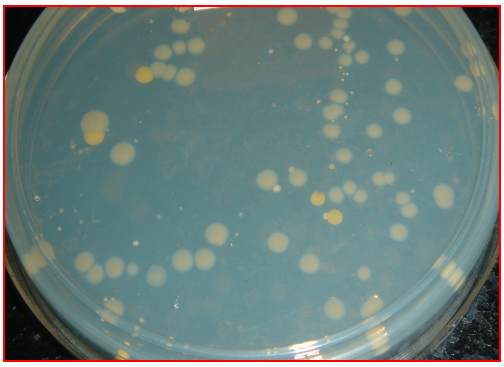
POSTTREATMENT



3RD DAY

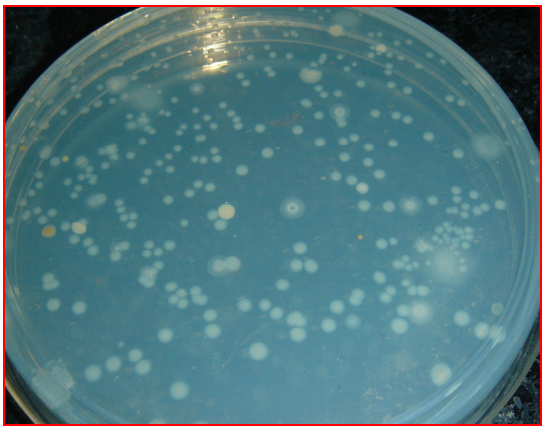


5TH DAY

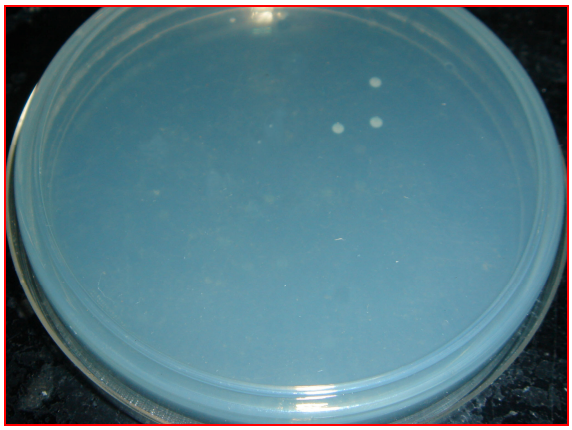


7TH DAY

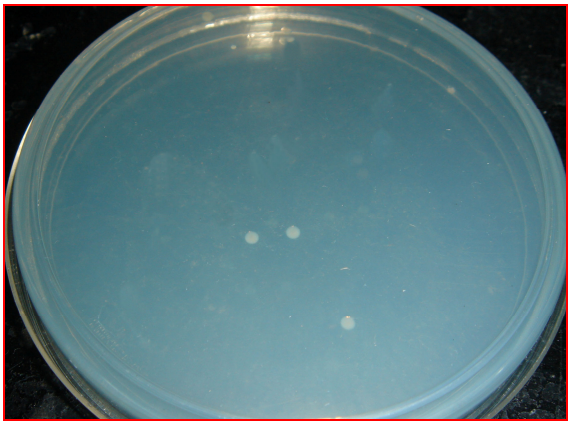
CULTURE PLATES SHOWING REDUCTION IN MICROBIAL COUNT WITH 10% POVIDONE IODINE



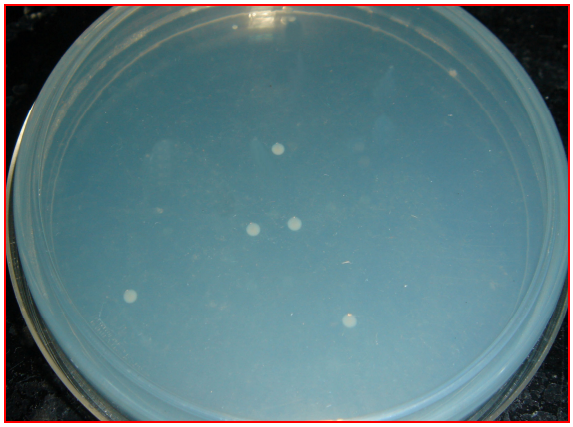
BASELINE



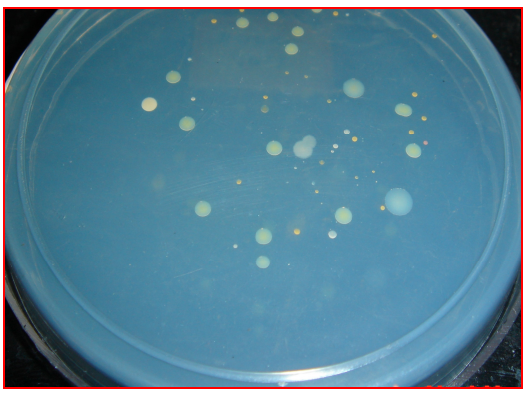
POST TREATMENT



3RD DAY



5TH DAY



7TH DAY